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(54) **SITE-SPECIFIC GLYCAN REMODELING OF LYSOSOMAL ENZYMES AND APPLICATIONS THEREOF**

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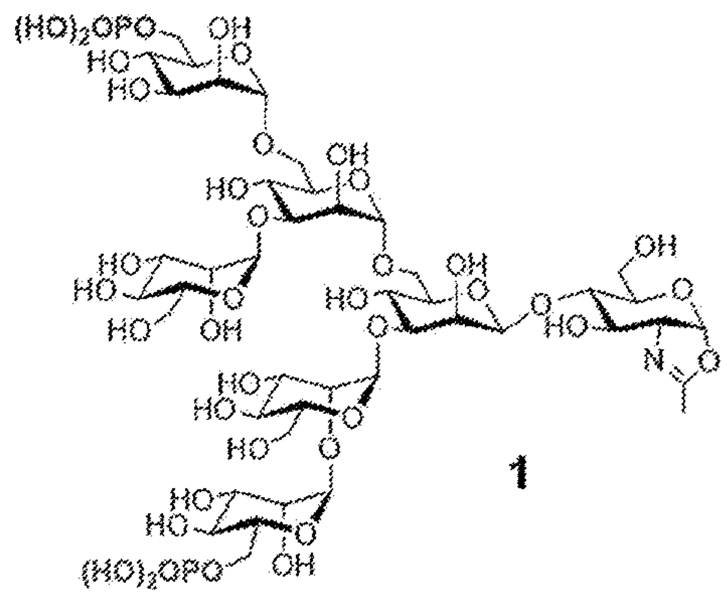
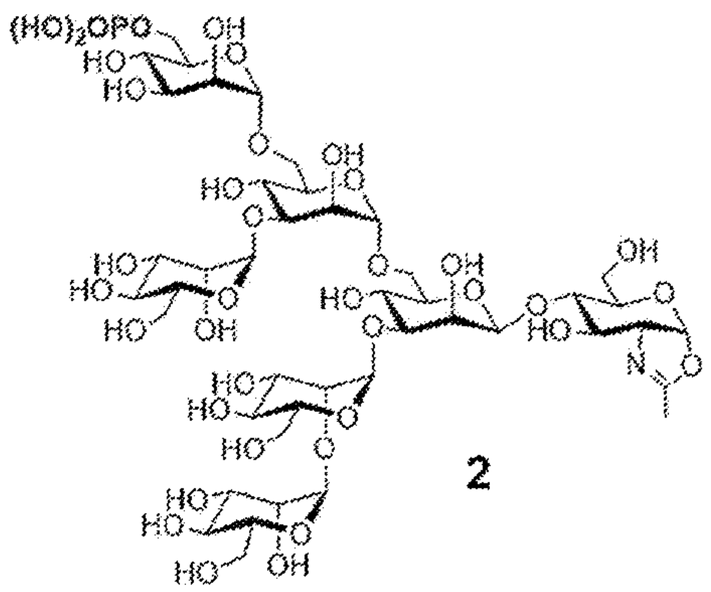
(57) **ABSTRACT**

The present disclosure provides compounds useful for enzymatic glycan remodeling of a glycoprotein. Also provided is a method for remodeling a glycoprotein using M6P-glycan oxazolines in a one-pot deglycosylation/transglycosylation process, which may enable site selective M6P-glycan remodeling of glycoproteins to obtain homogeneous products. The remodeled glycoprotein (such as a recombinant human acid α-glucosidase) may have enhanced affinity for the CI-MPR, increased uptake by a cell, and improved therapeutic efficacy compared to the original glycoprotein. A method of treating Pompe disease using a glycan remodeled lysosomal enzyme is also provided.

Specification includes a Sequence Listing.



1



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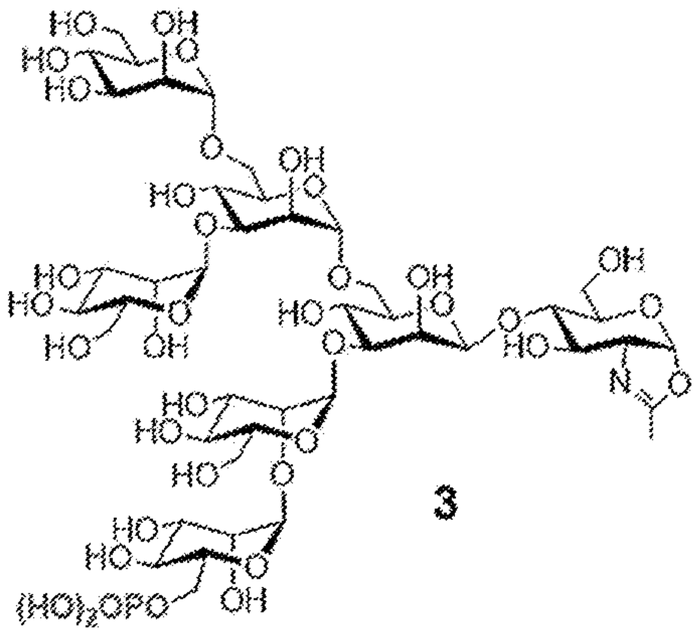

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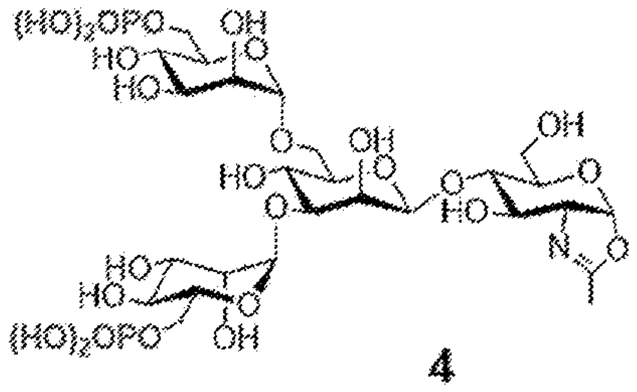

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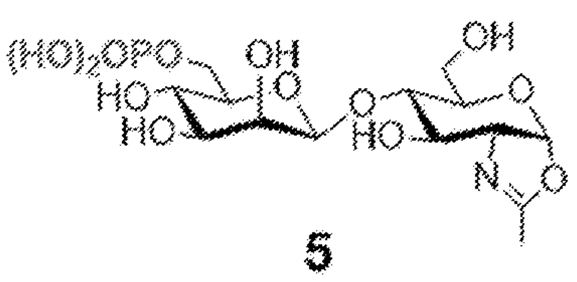


5

FIG. 1

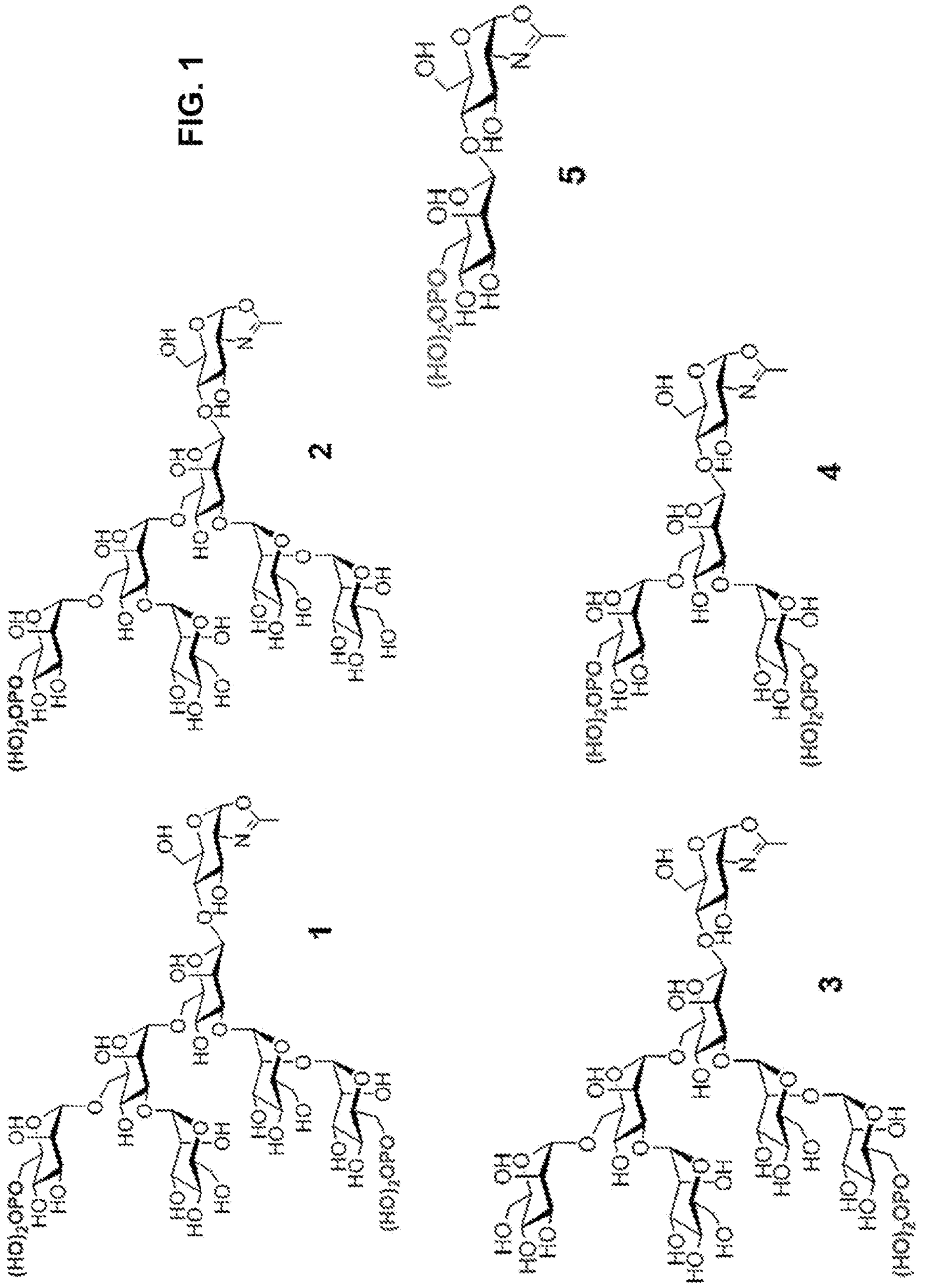
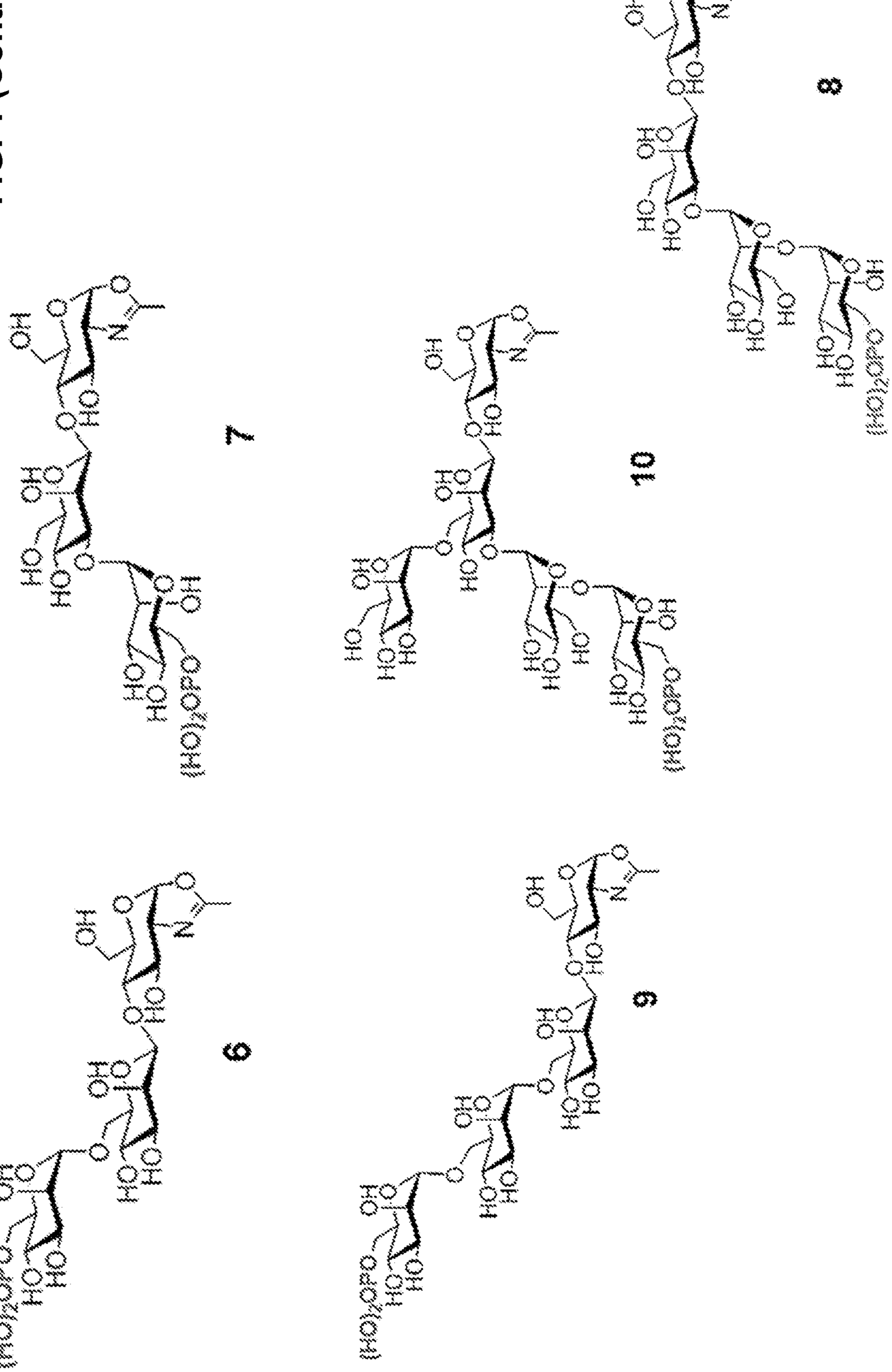


FIG. 1 (Cont.)



8

10

7

9

6

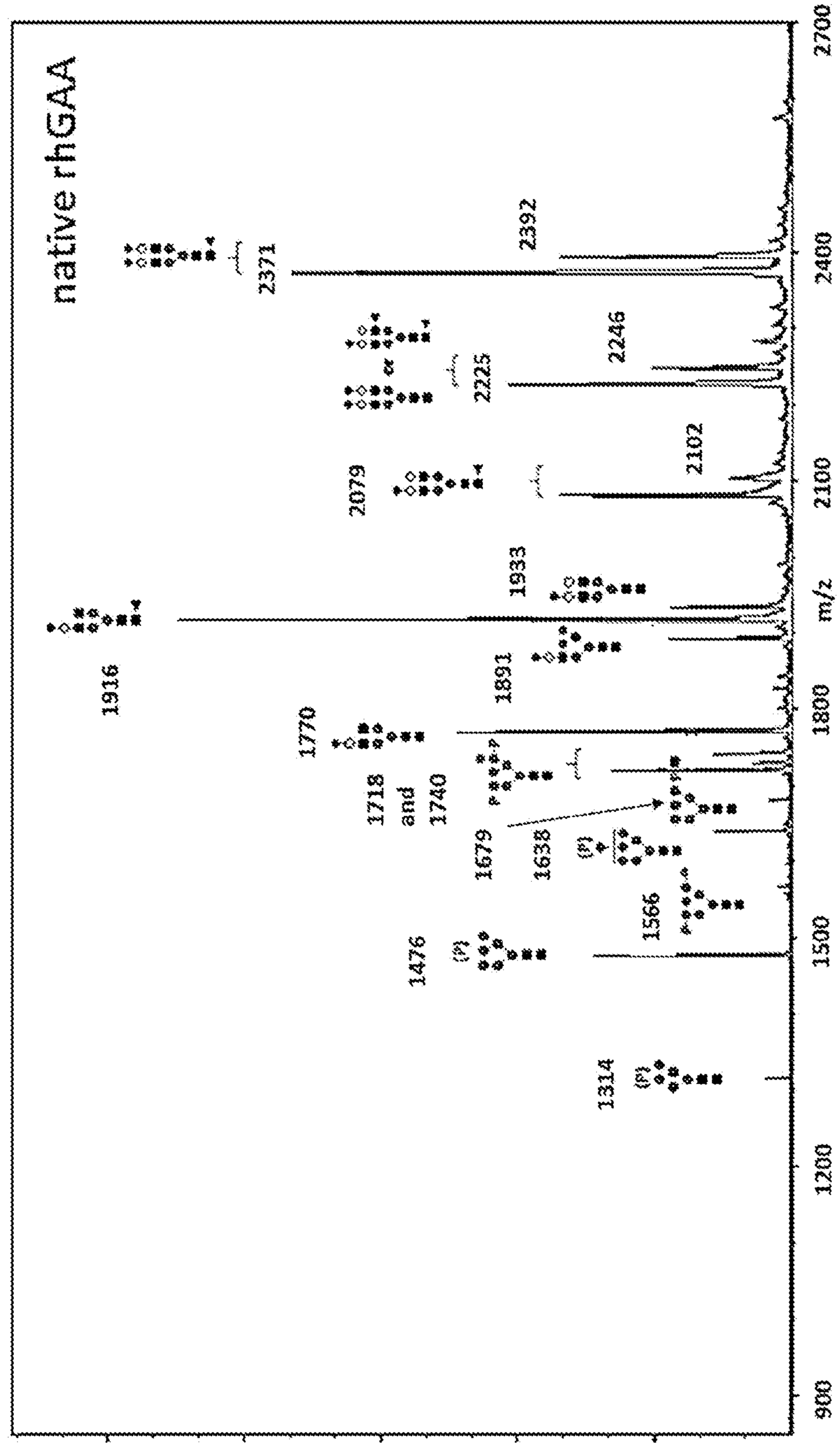
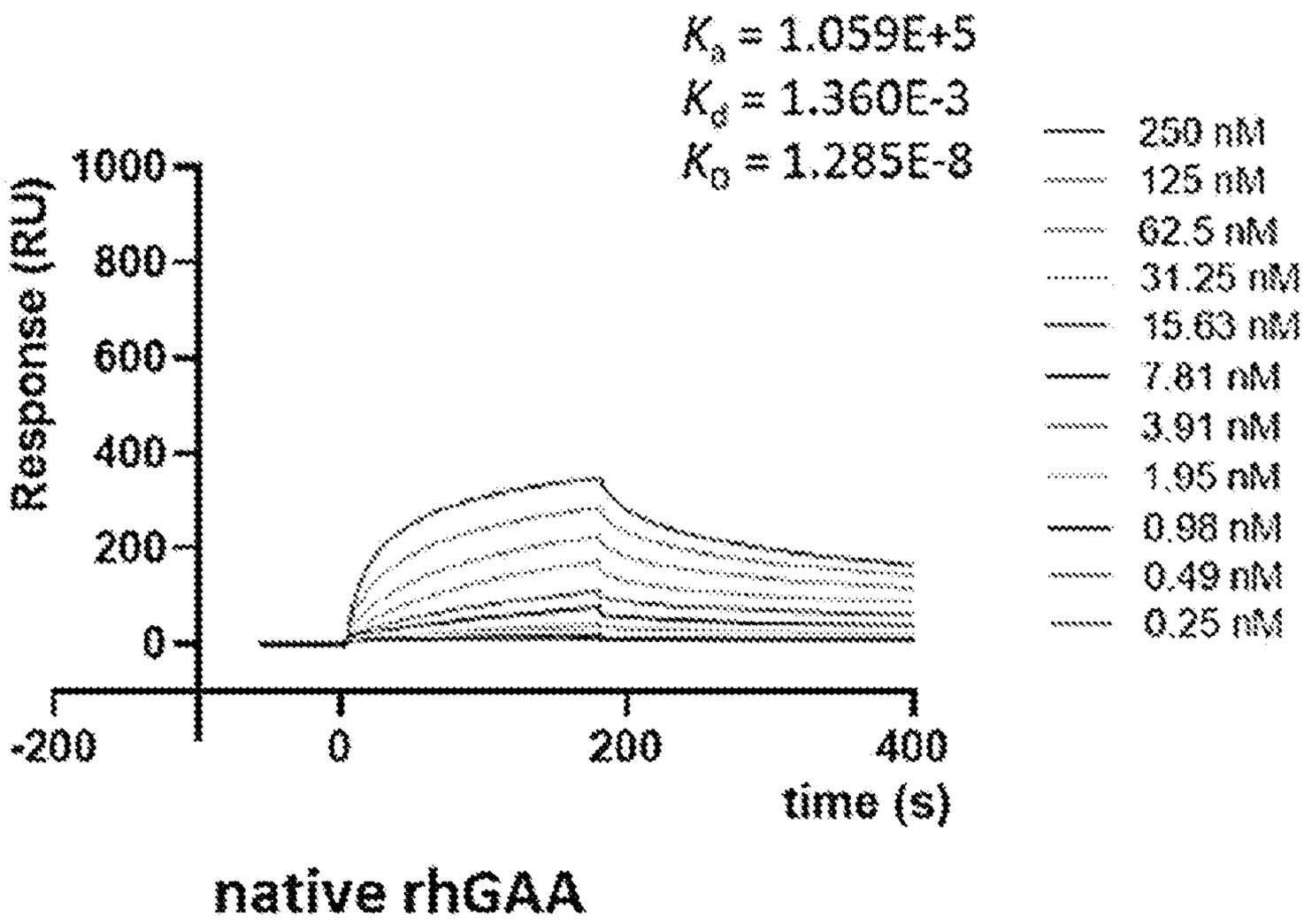

native rhGAA
15000
12000
9000
6000
3000
900
1200
1500
1800
2100
2700
m/z
1314
1566
1638
1679
1718
and
1740
1770
1891
1916
1933
2079
2102
2225
2246
2371
2392
2400
(P)
(P)
(P)

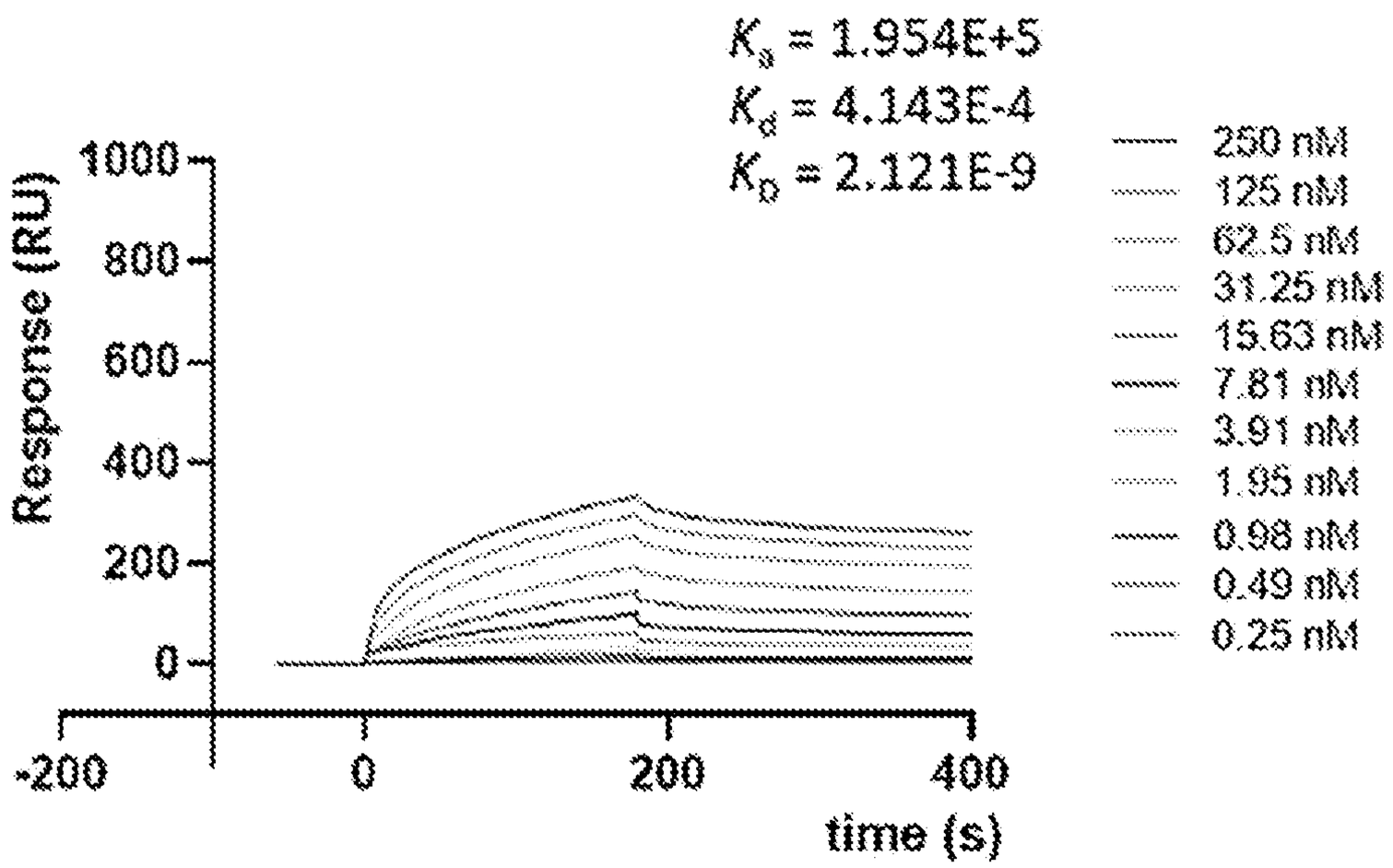
FIG. 2A

FIG. 2B



native rhGAA

FIG. 2D



Endo A-remodeled rhGAA

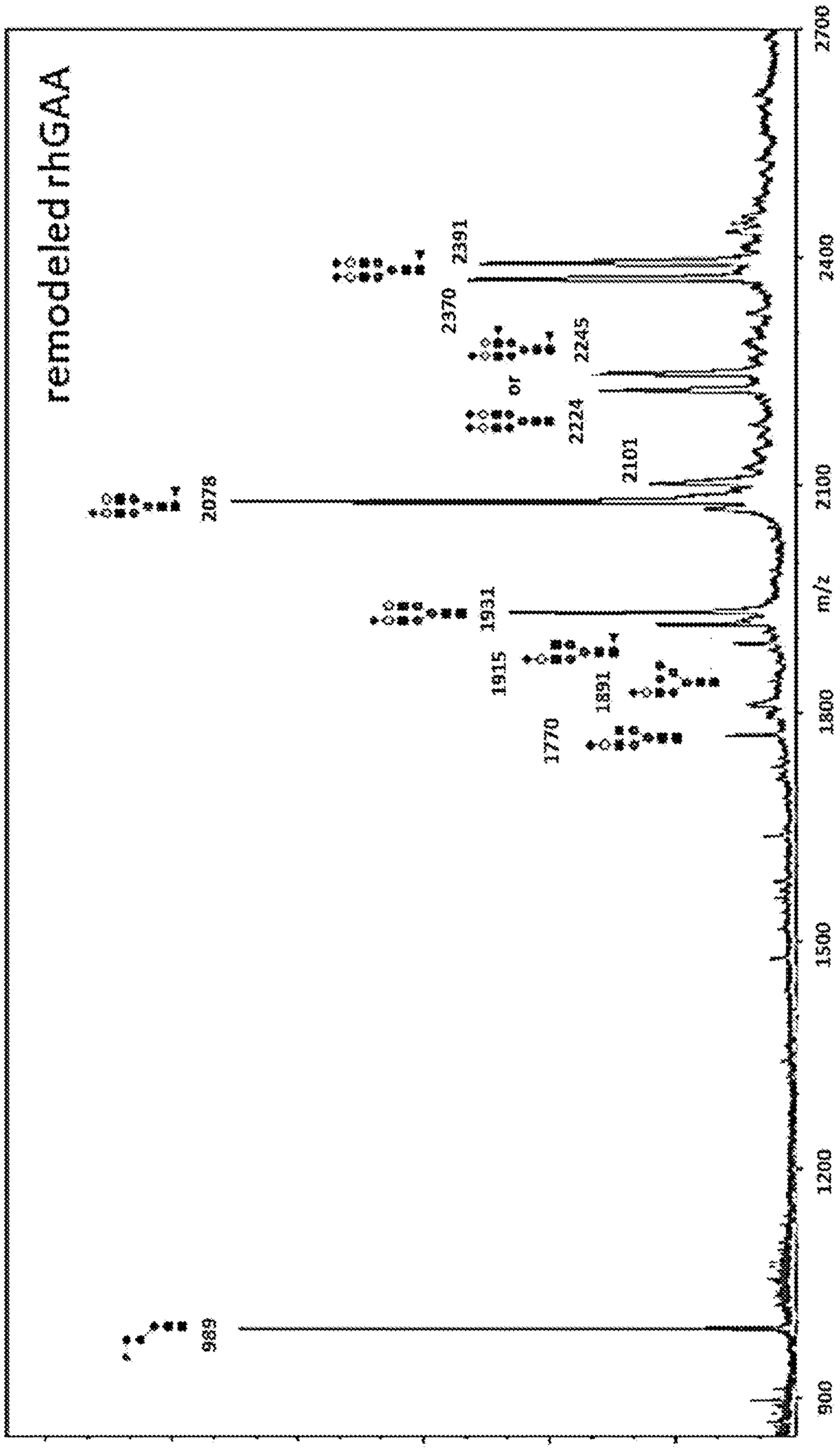

remodeled rhGAA
18000
15000
12000
9000
6000
3000
989
900
1200
2078
1500
1770
1800
1891
1915
1931
m/z
2100
2101
2224
or
2245
2370
2391
2400
2708

FIG. 2C

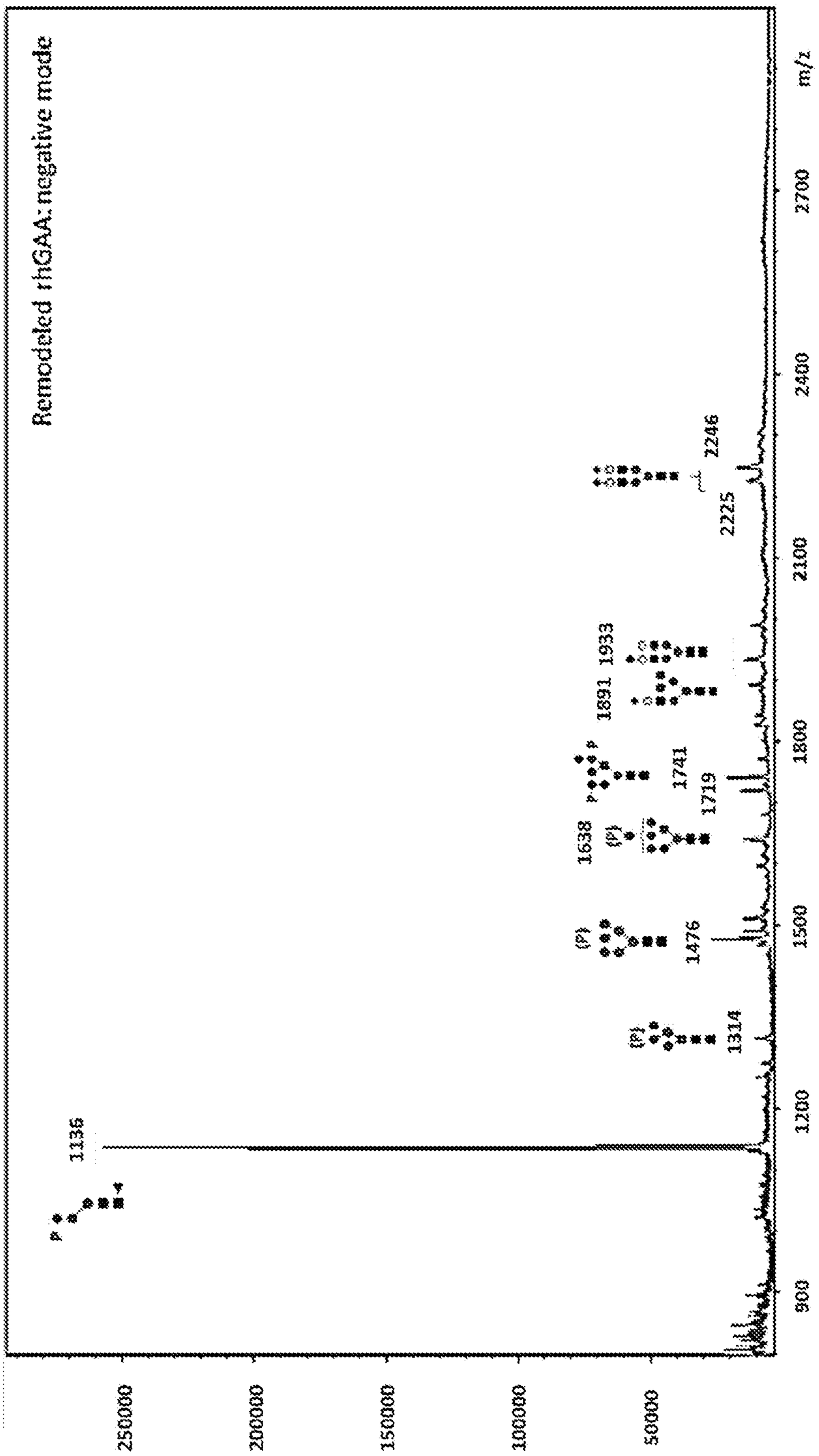
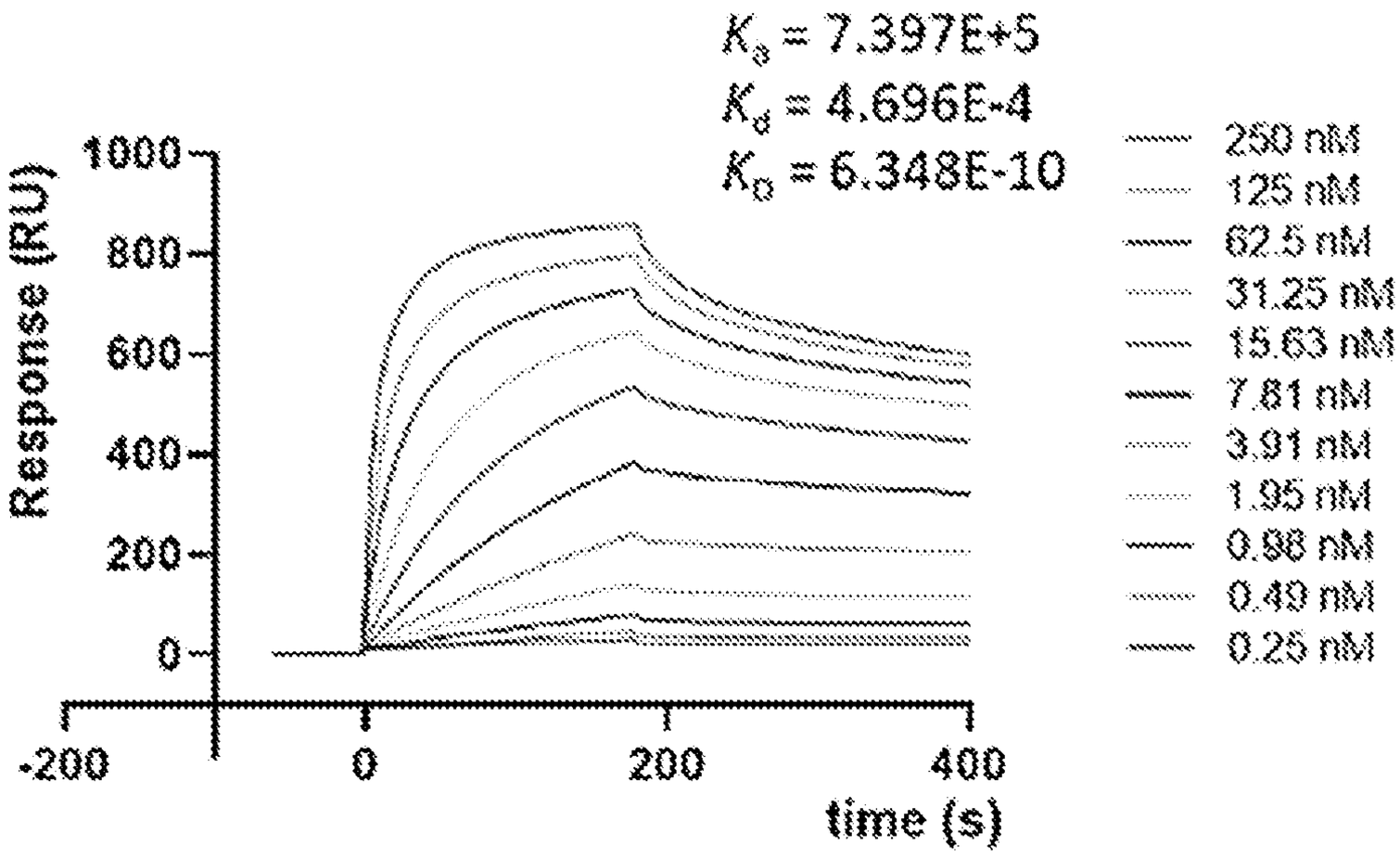

Remodeled rhGAA: negative mode
m/z
2700
2400
2246
2225
1891
1933
1638
1741
1719
1476
1500
1800
2100
1136
1314
1200
900
250000
200000
150000
100000
50000

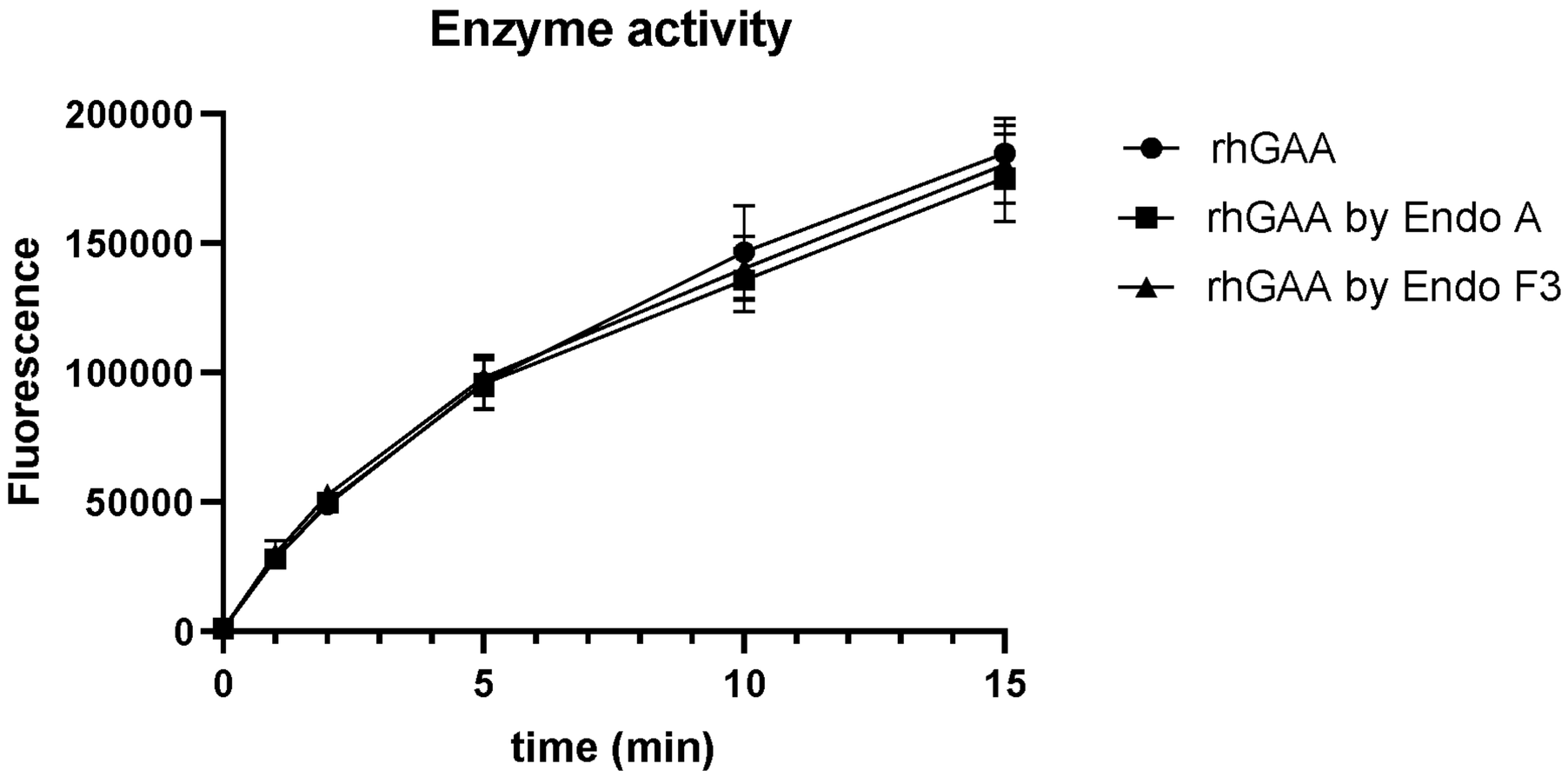
FIG. 2E

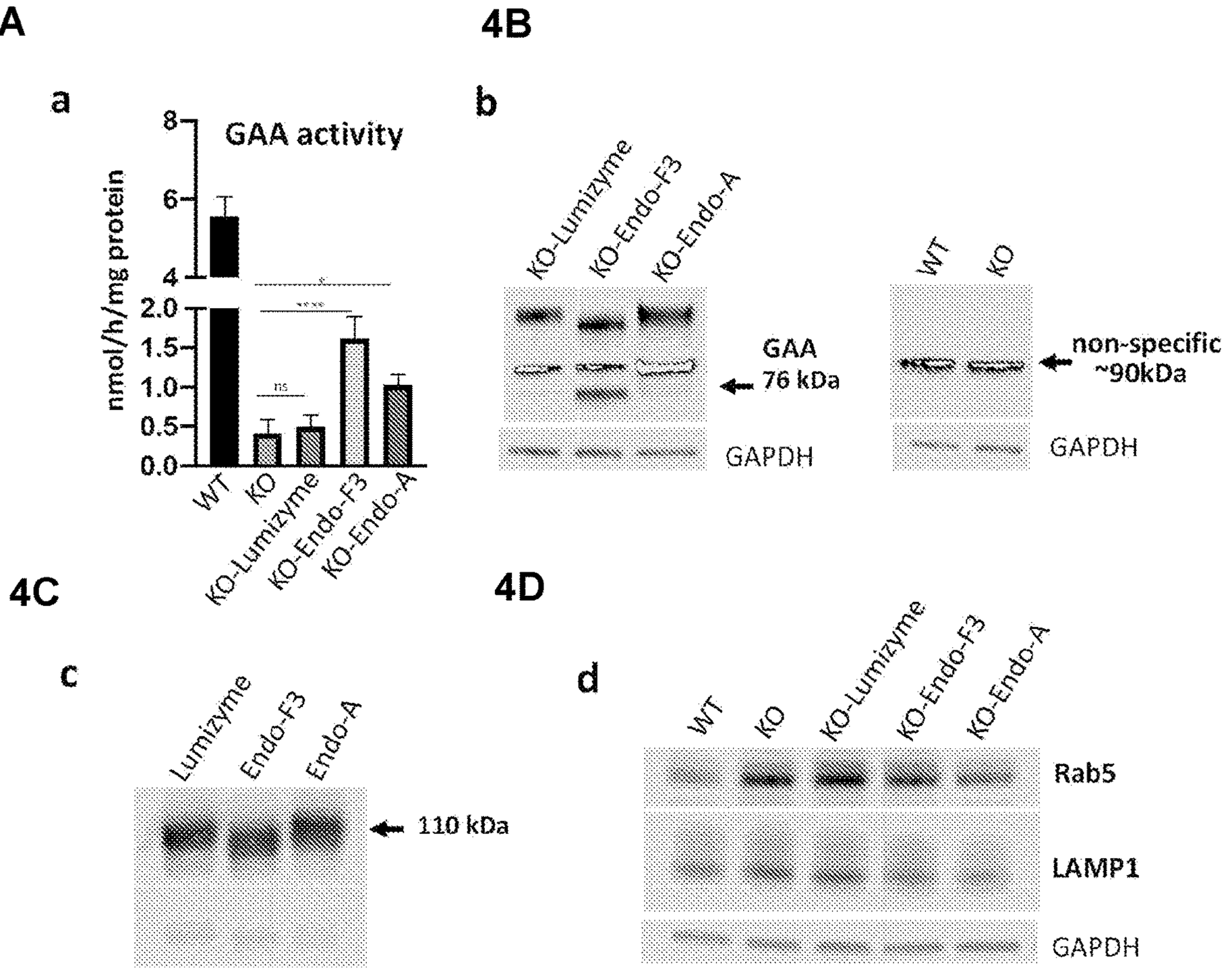
FIG. 2F



Endo F3-remodeled rhGAA

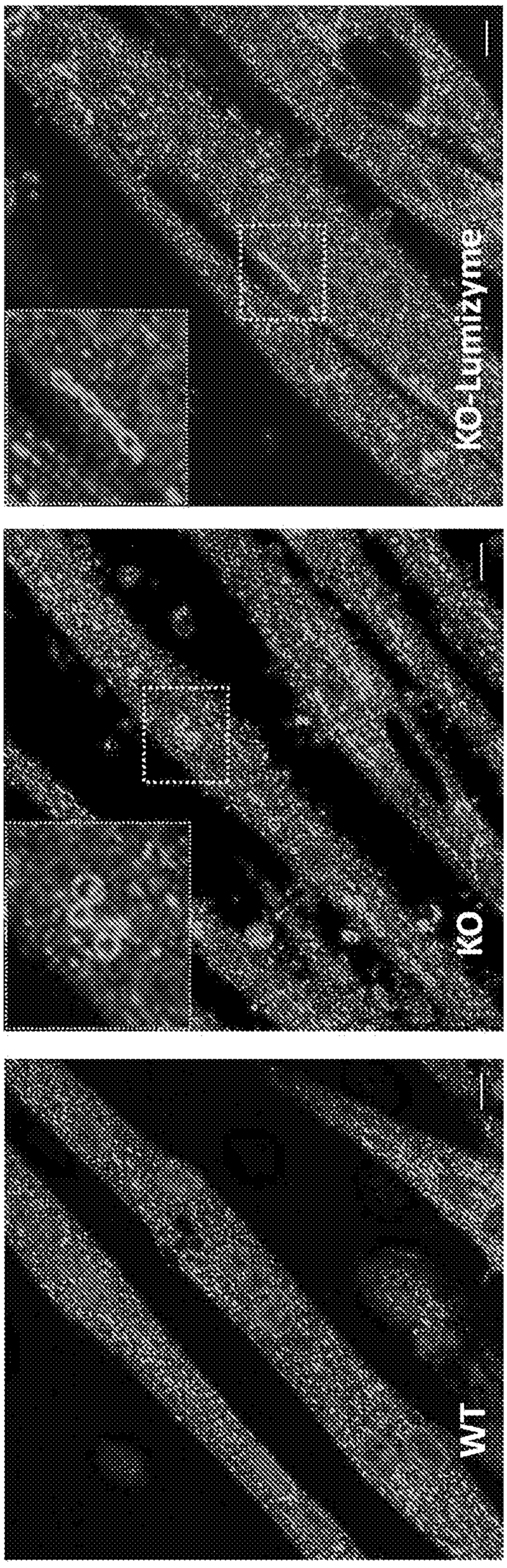
FIG. 3




4A
4B
a
GAA activity
nmol/h/mg protein
8
6
4
2.0
1.5
1.0
0.5
0.0
ns
****
WT
KO
KO-Lumizyme
KO-Endo-F3
KO-Endo-A
b
KO-Lumizyme
KO-Endo-F3
KO-Endo-A
GAA
76 kDa
GAPDH
WT
KO
non-specific
~90kDa
GAPDH
4C
4D
c
Lumizyme
Endo-F3
Endo-A
110 kDa
d
WT
KO
KO-Lumizyme
KO-Endo-F3
KO-Endo-A
Rab5
LAMP1
GAPDH

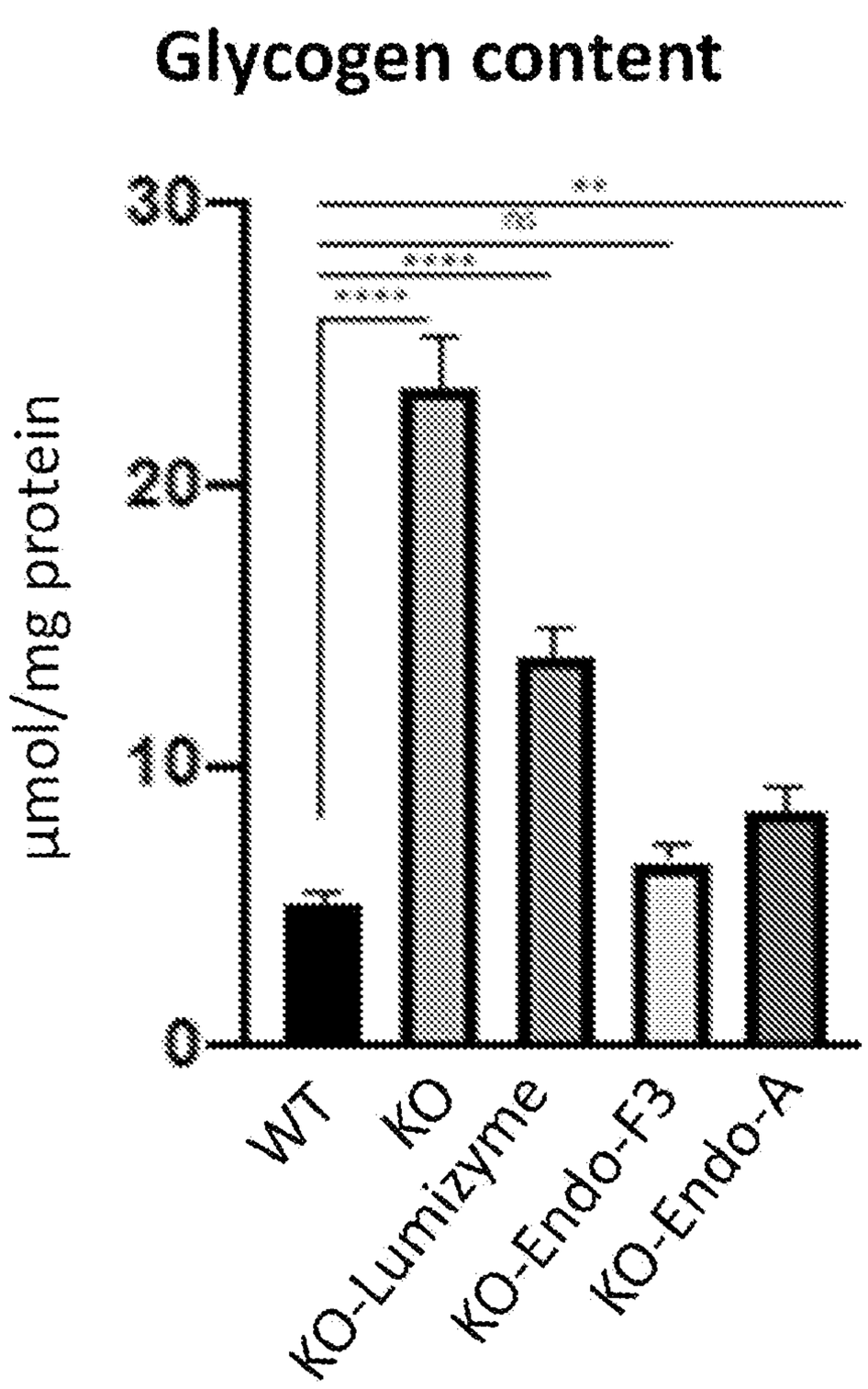
FIGS. 4A-4D

5A

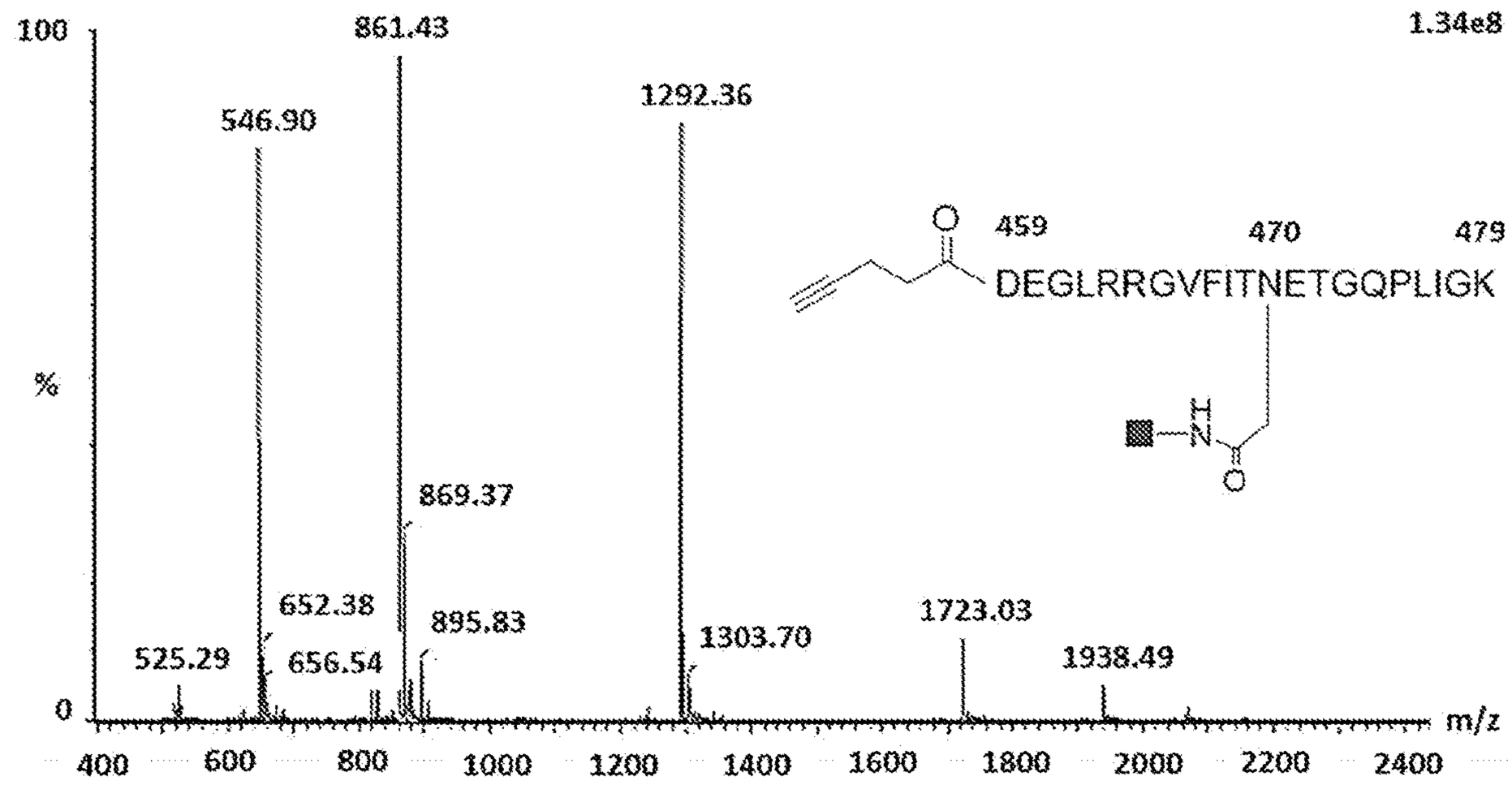


FIGS. 5A-5B

5B



FIGS. 5A-5B (Cont.)


100
%
0
861.43
546.90
1292.36
869.37
652.38
895.83
525.29
656.54
1303.70
1723.03
1938.49
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
m/z
1.34e8
459
470
479
DEGLRRGVFITNETGQPLIGK

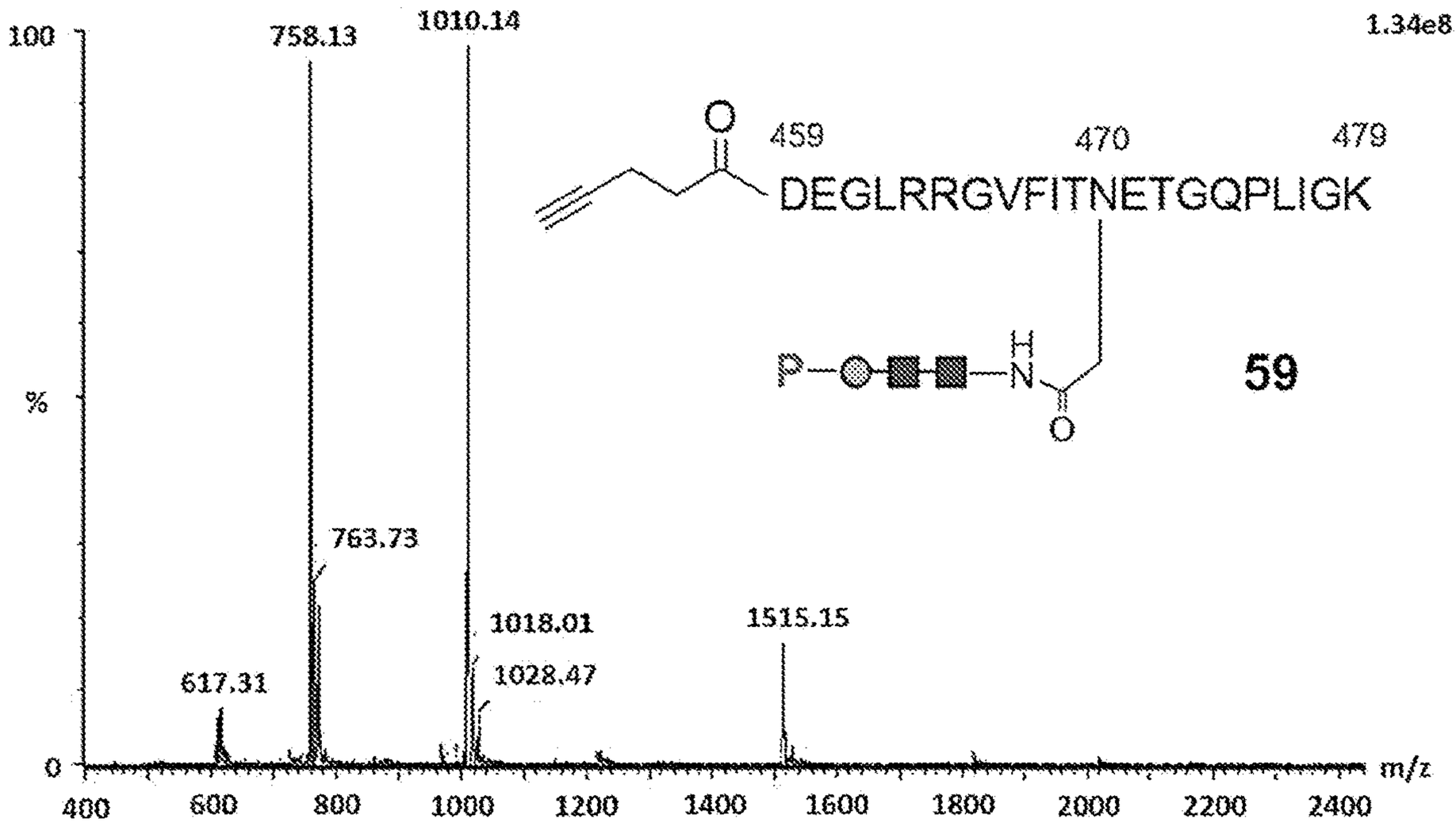
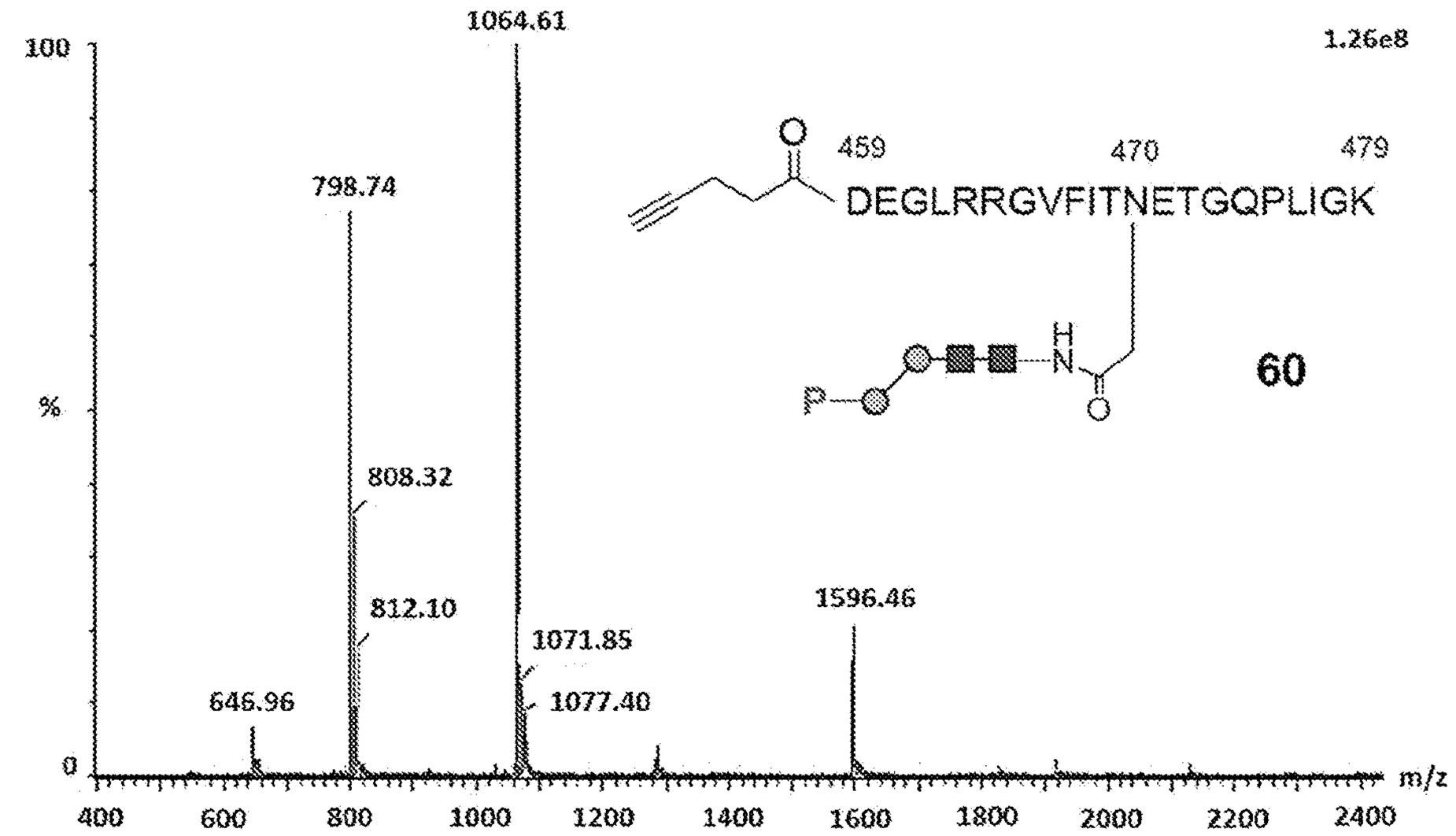

100
%
0
758.13
1010.14
763.73
1018.01
1515.15
617.31
1028.47
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
m/z
1.34e8
459
470
479
DEGLRRGVFITNETGQPLIGK
59

FIG. 6



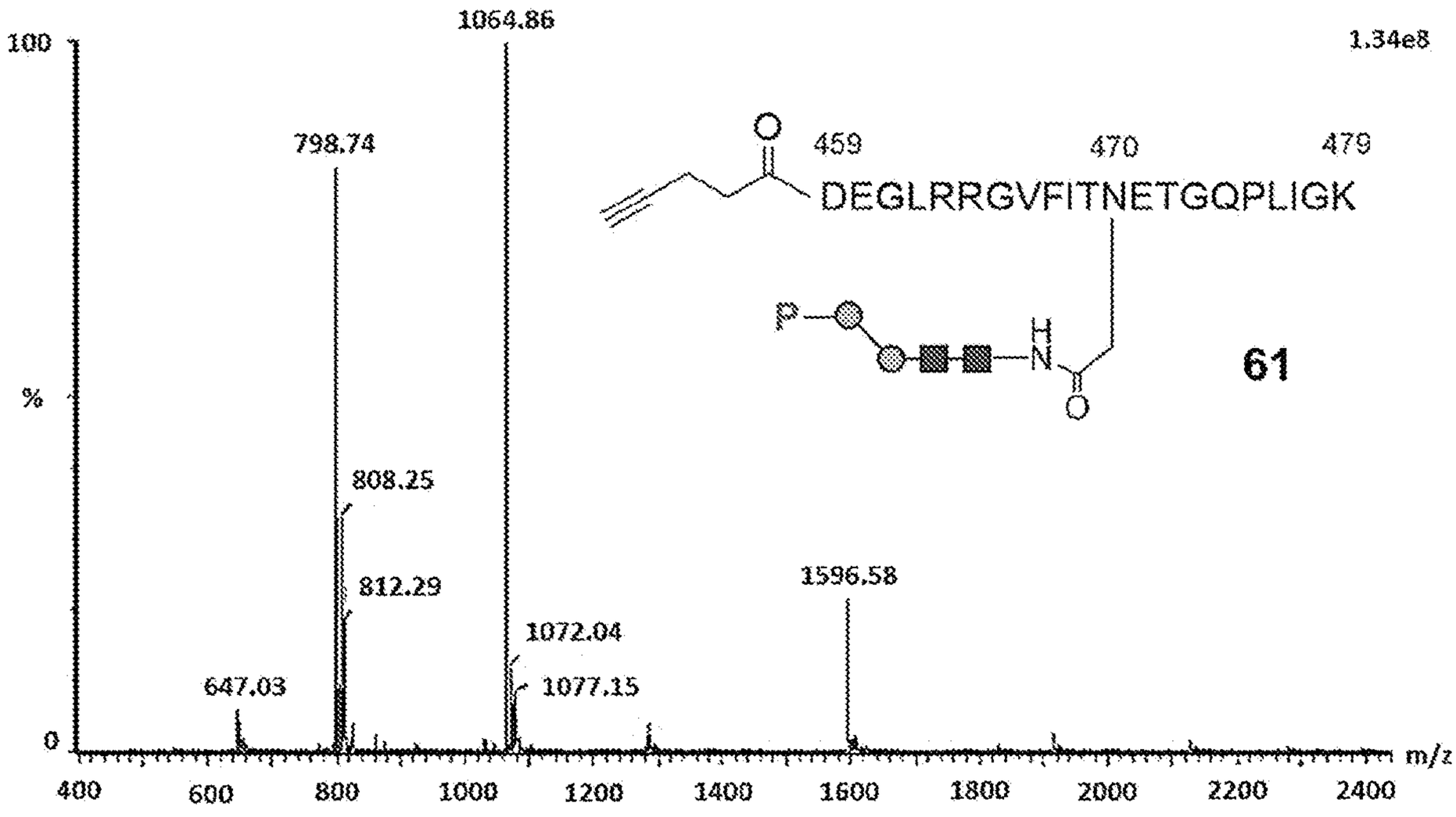
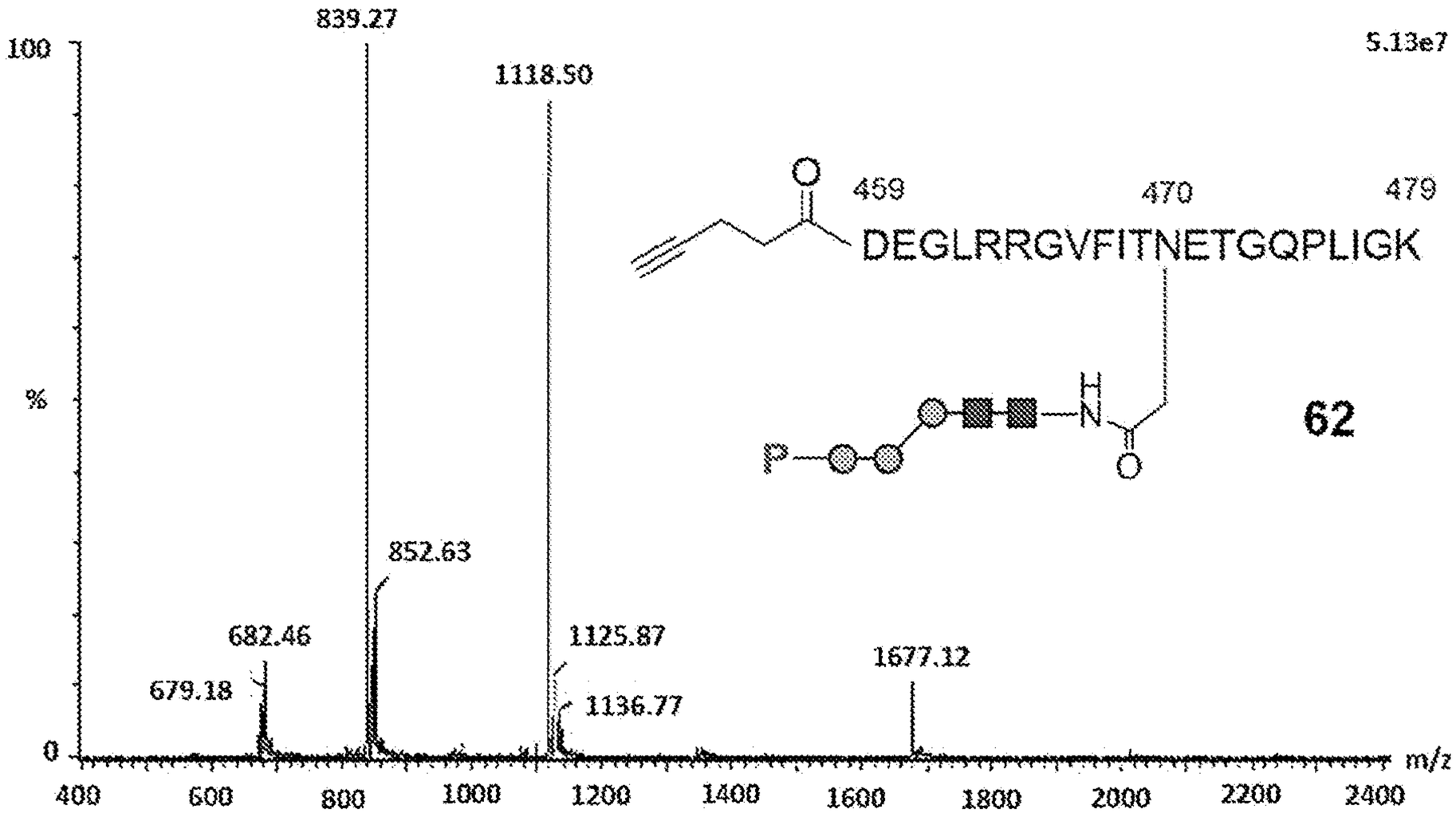


FIG. 6 (continued)



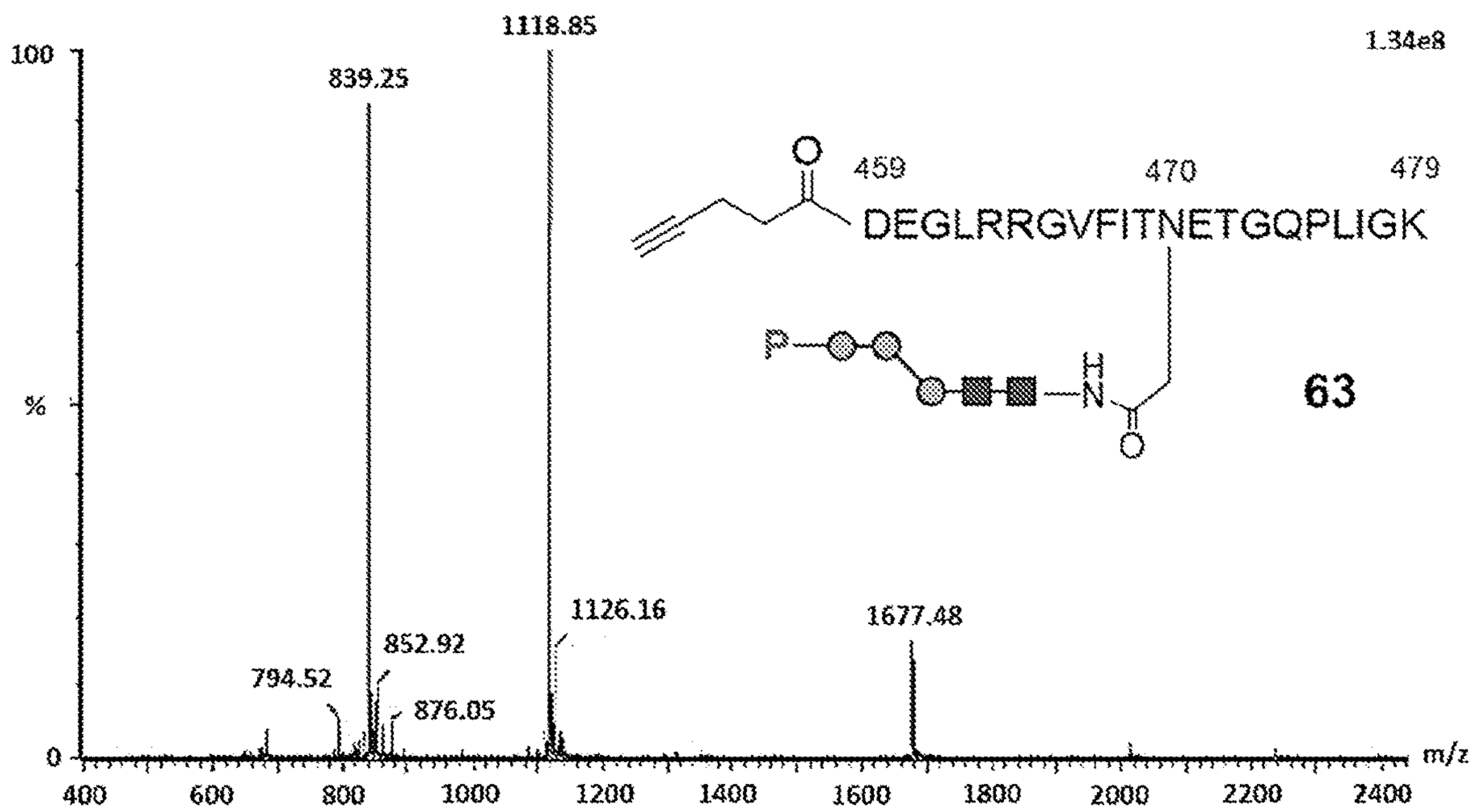
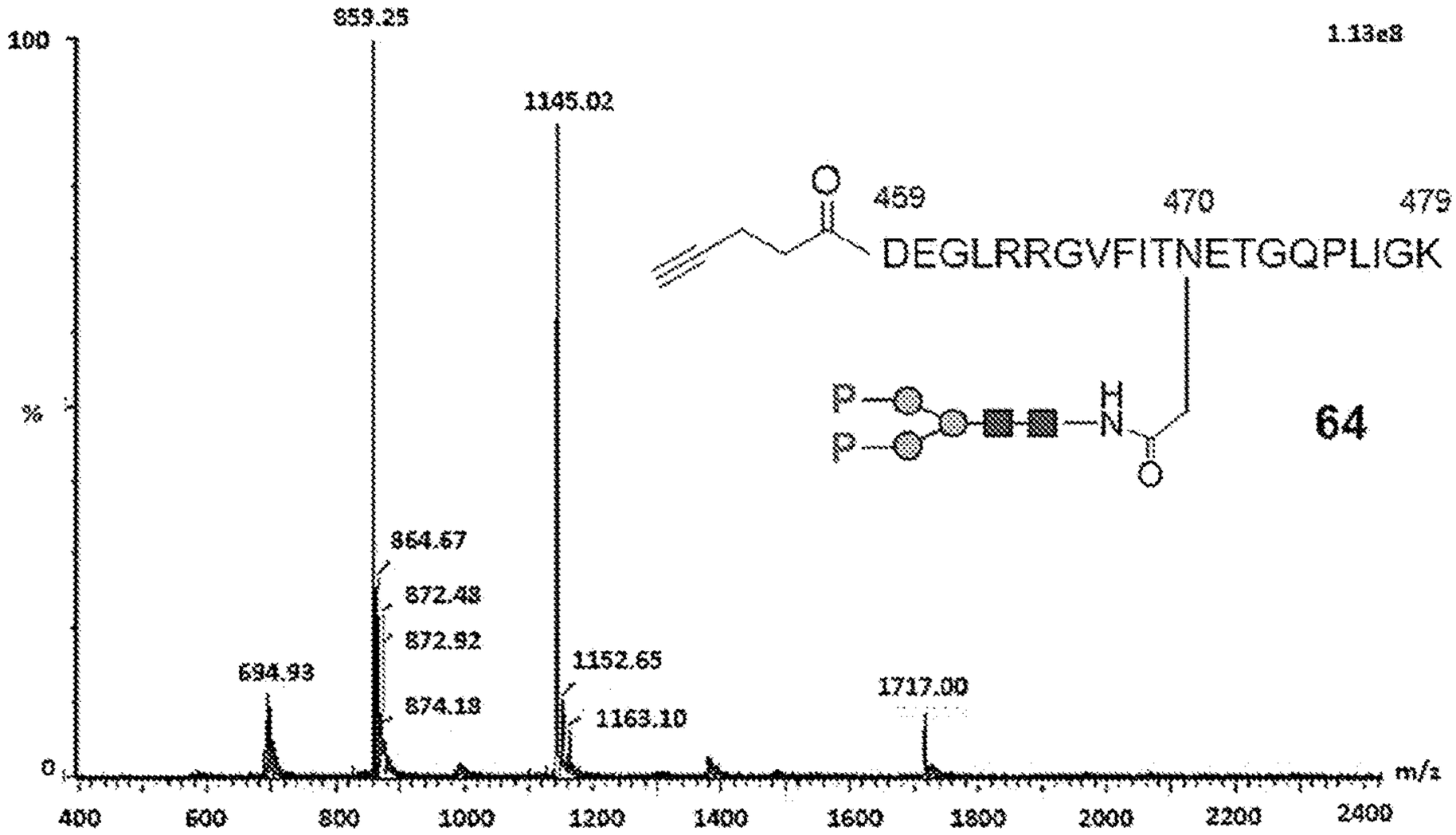


FIG. 6 (continued)



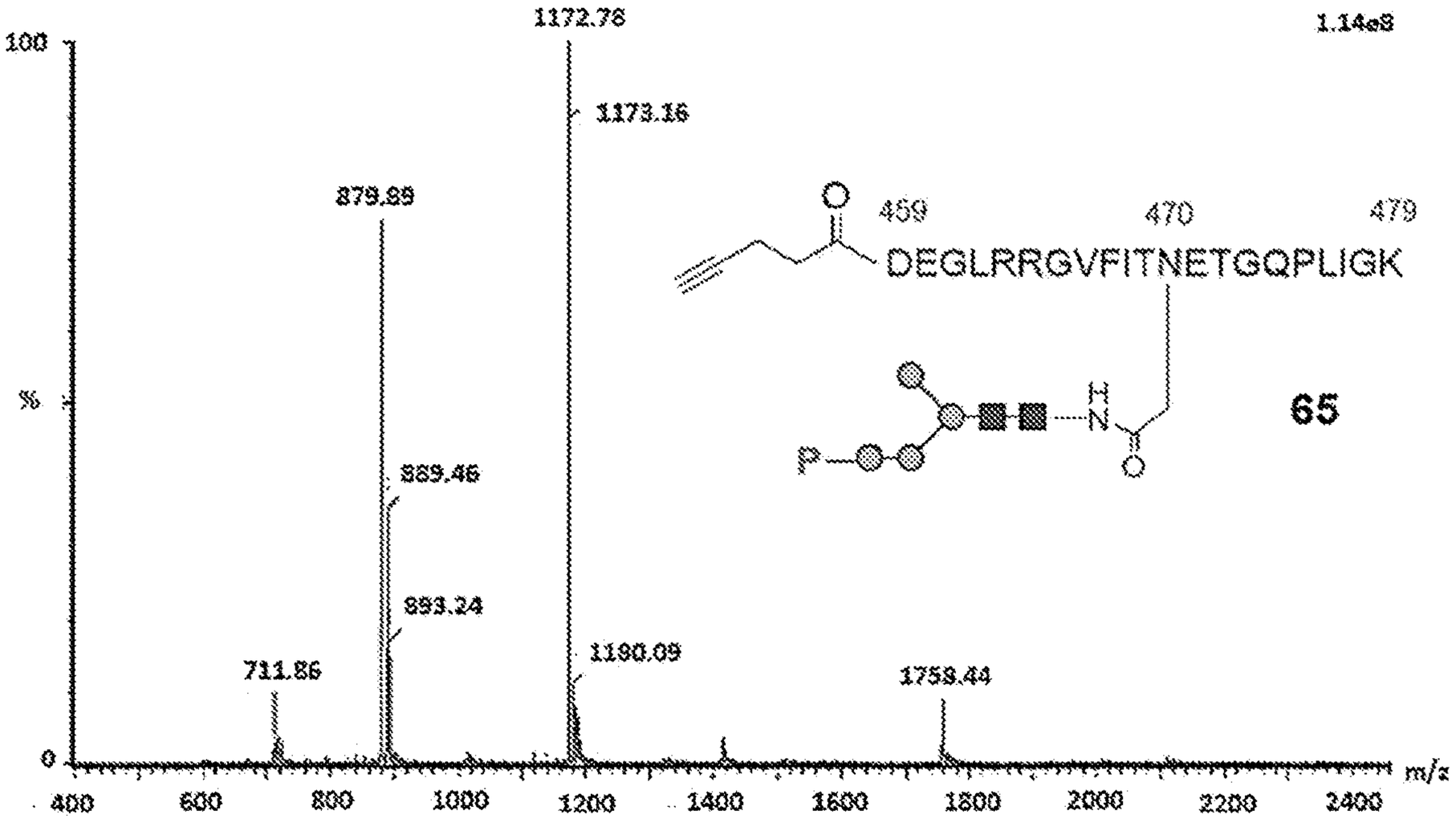


FIG. 6 (continued)

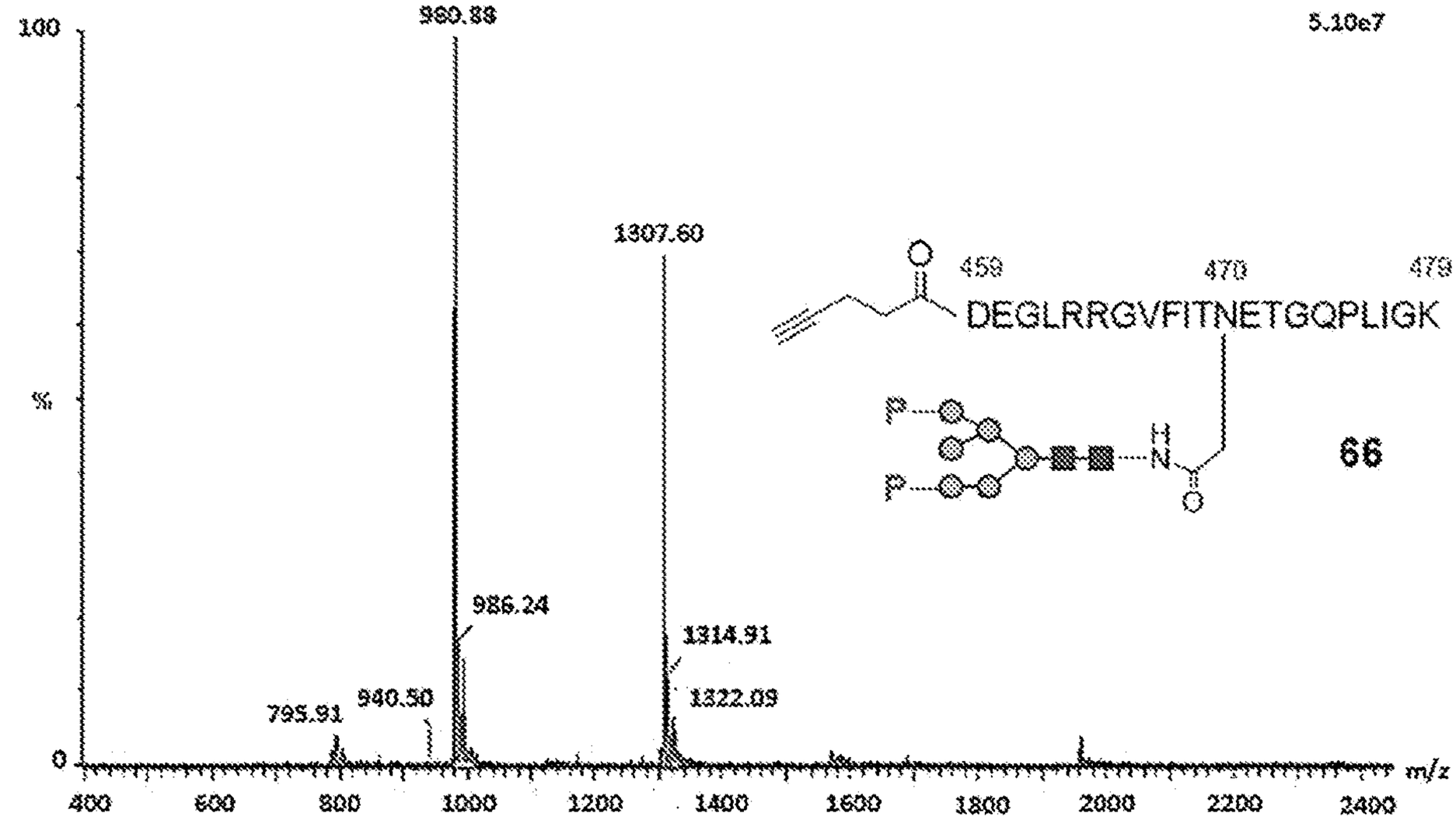


FIG. 6 (continued)

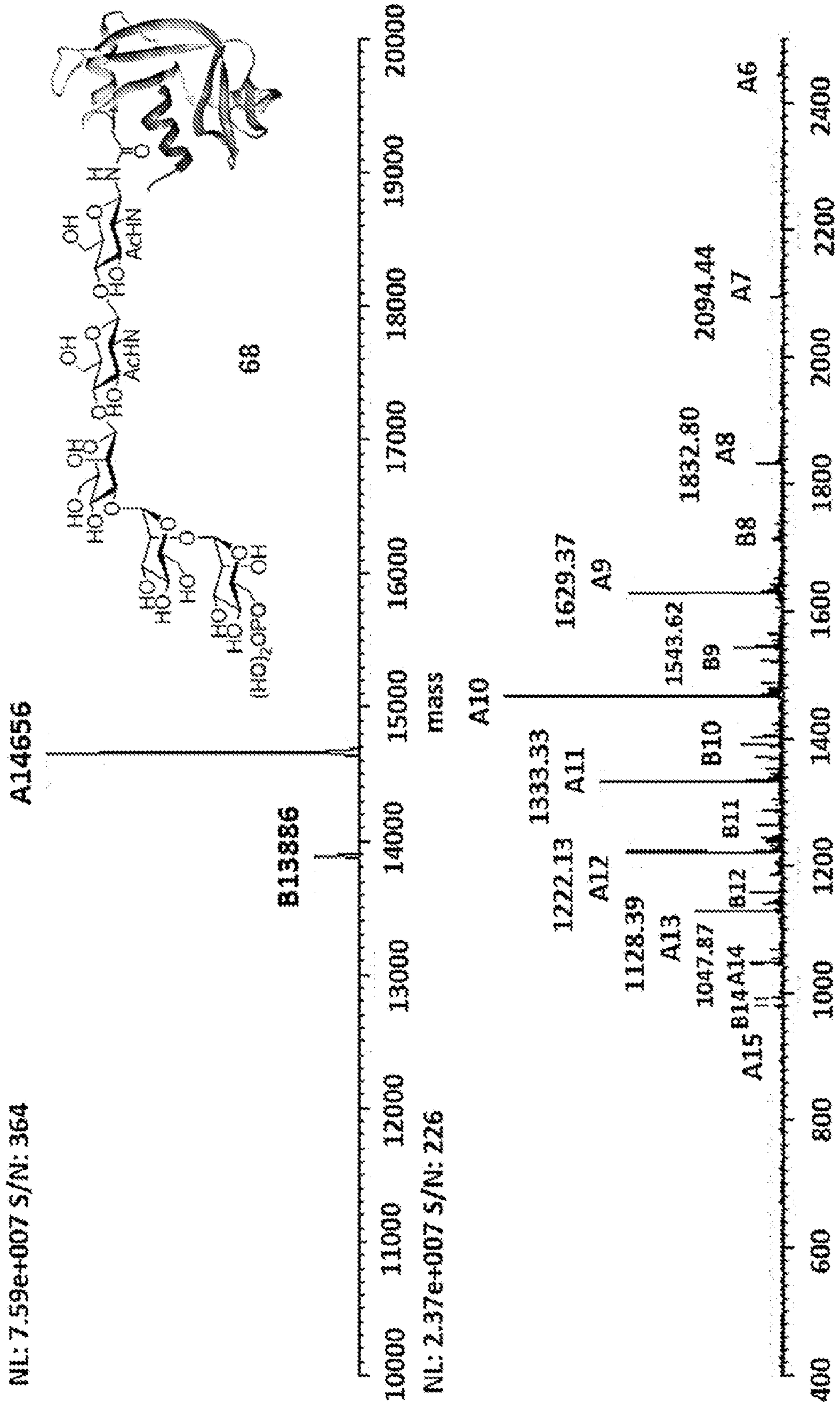


FIG. 7

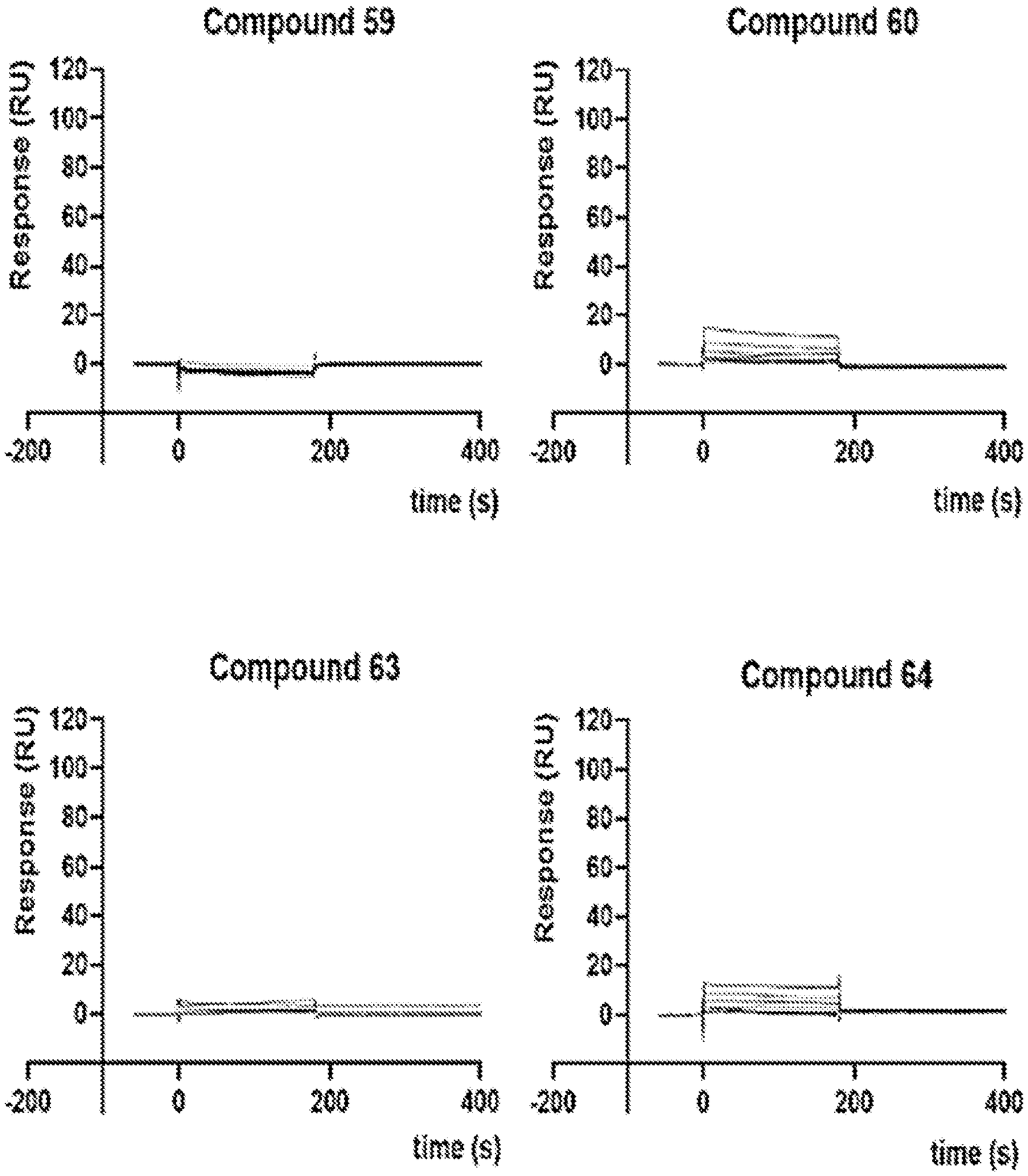
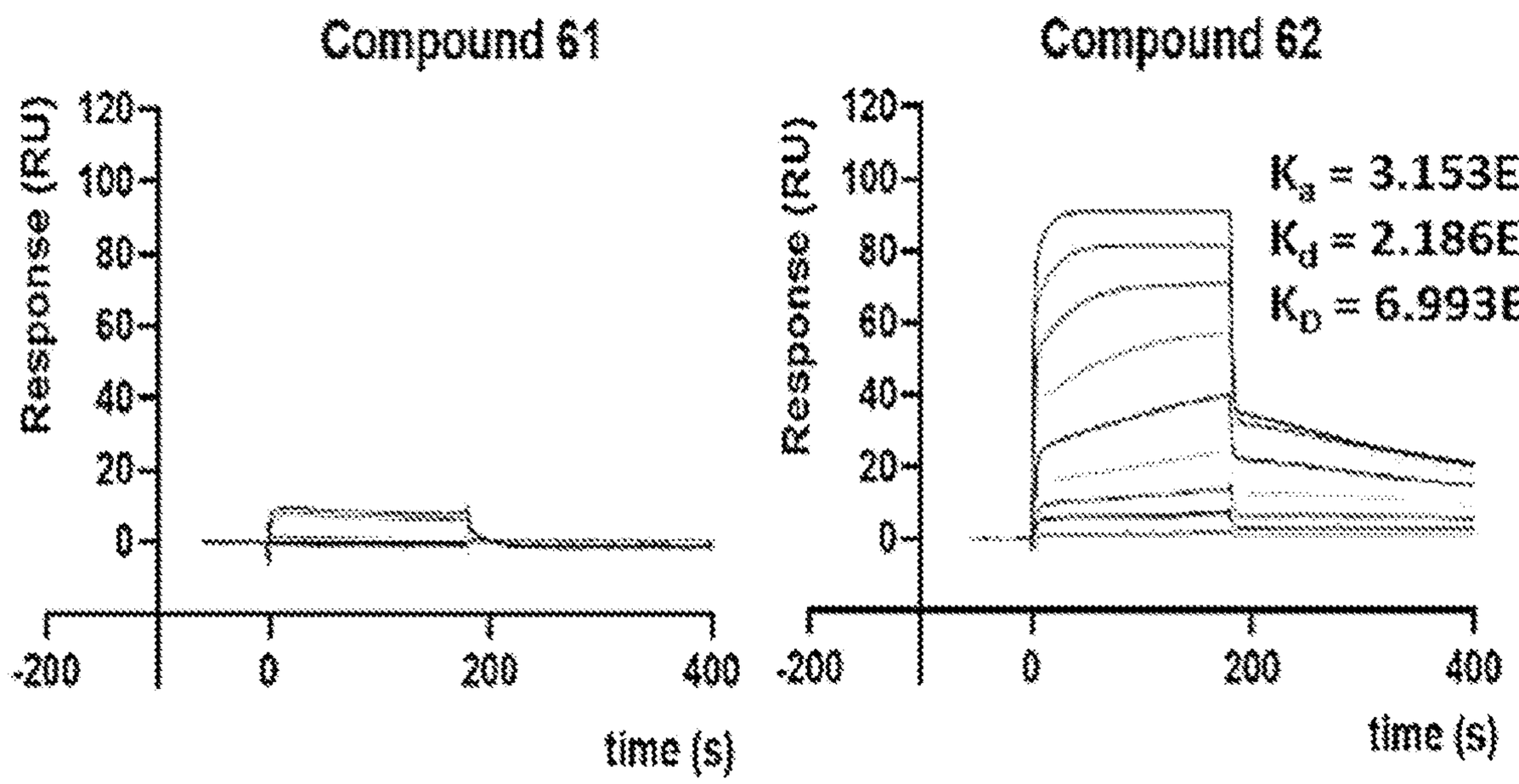

Compound 59
Response (RU)
120
100
80
60
40
20
0
-200
0
200
400
time (s)
Compound 60
Response (RU)
120
100
80
60
40
20
0
-200
0
200
400
time (s)
Compound 63
Response (RU)
120
100
80
60
40
20
0
-200
0
200
400
time (s)
Compound 64
Response (RU)
120
100
80
60
40
20
0
-200
0
200
400
time (s)

FIG. 8


Compound 61
Response (RU)
120
100
80
60
40
20
0
-200
0
200
400
time (s)
Compound 62
Response (RU)
K_a = 3.153E+4
K_d = 2.186E-3
K_D = 6.993E-8
time (s)

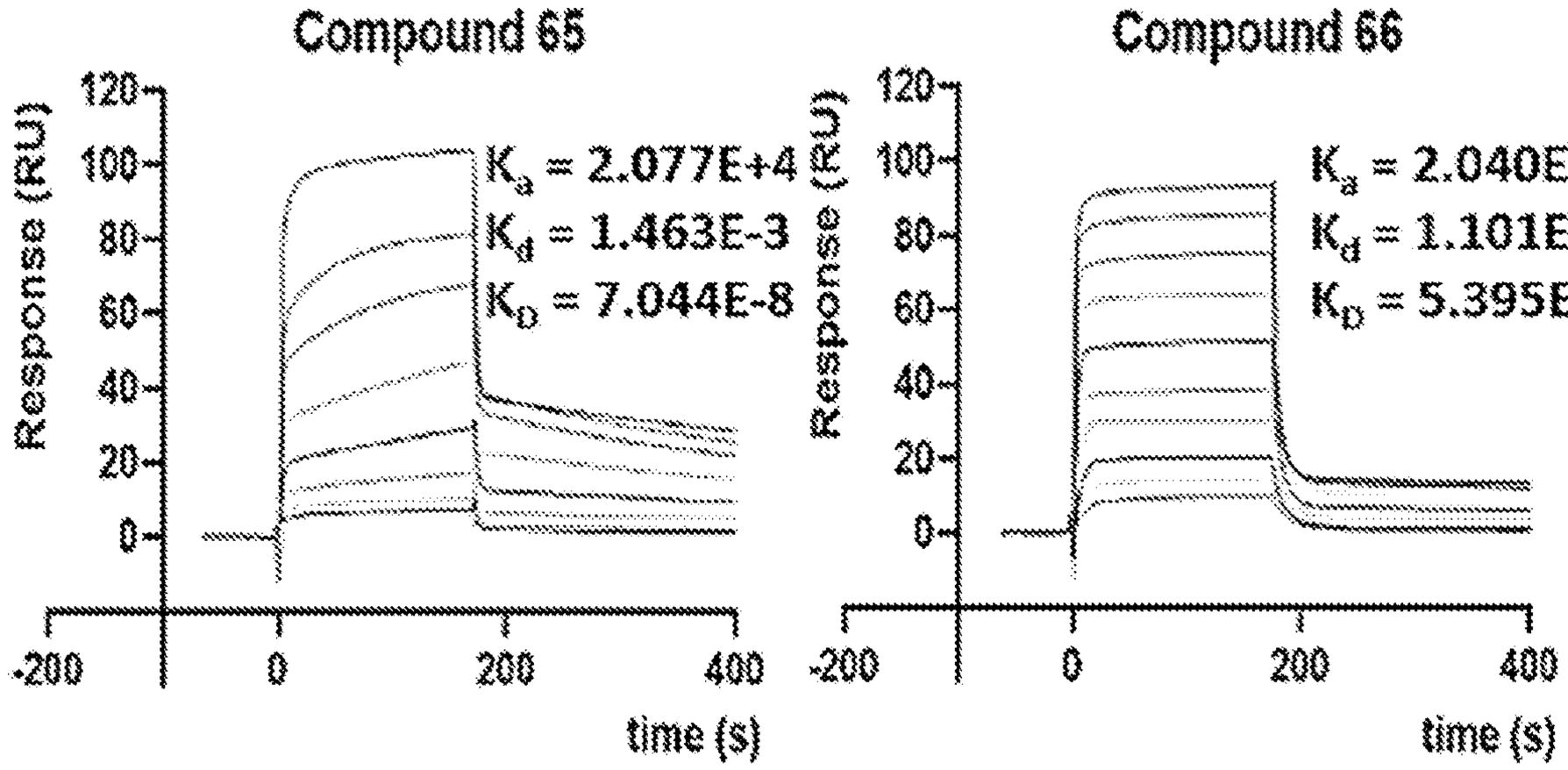

Compound 65
Response (RU)
K_a = 2.077E+4
K_d = 1.463E-3
K_D = 7.044E-8
time (s)
Compound 66
Response (RU)
K_a = 2.040E+4
K_d = 1.101E-3
K_D = 5.395E-8
time (s)

FIG. 8 (Cont.)

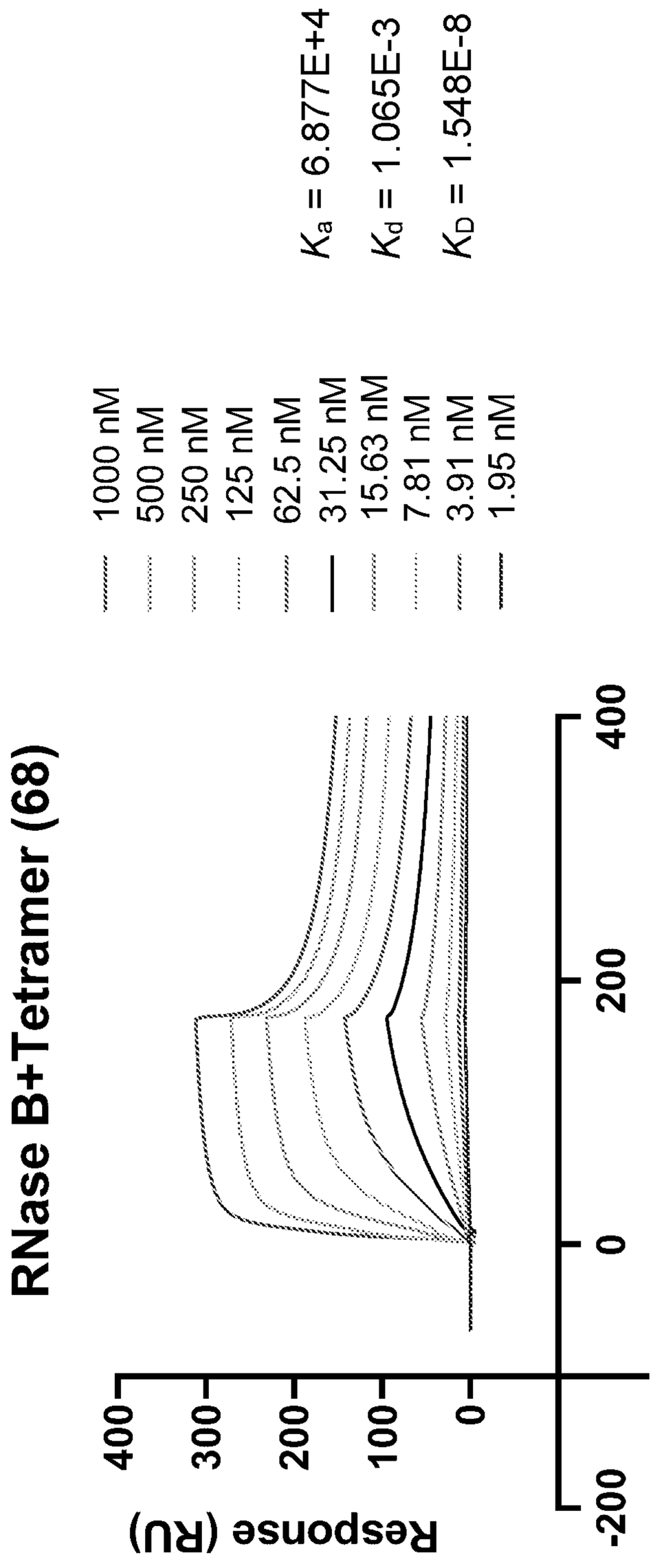

RNase B+Tetramer (68)
K_a = 6.877E+4
K_d = 1.065E-3
K_D = 1.548E-8
1000 nM
500 nM
250 nM
125 nM
62.5 nM
31.25 nM
15.63 nM
7.81 nM
3.91 nM
1.95 nM
Response (RU)
400
300
200
100
0
-200
0
200
400
time (s)

FIG. 9

FIG. 10A

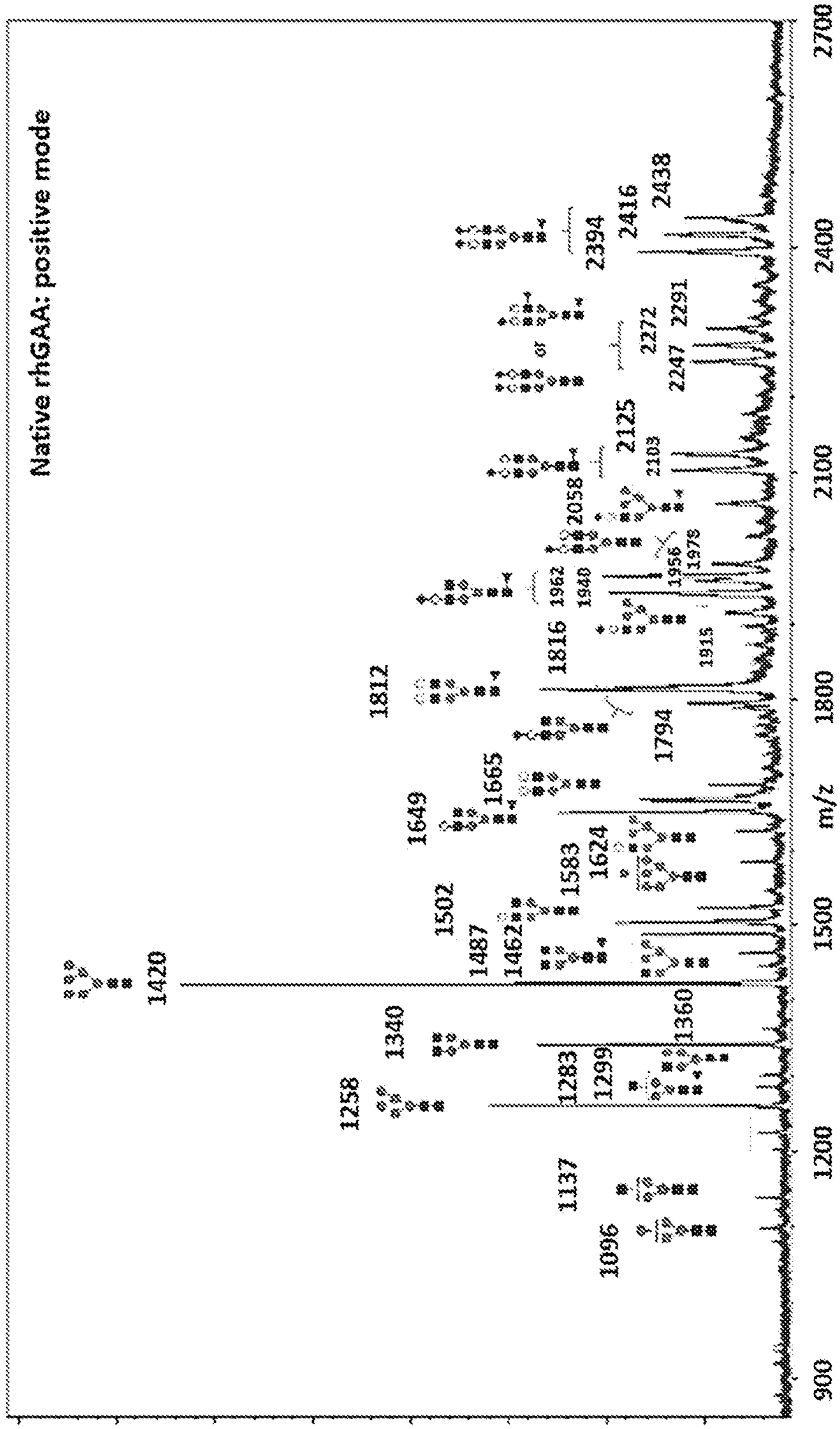

Native rhGAA: positive mode
8000
7000
6000
5000
4000
3000
2000
1000
900
1200
1500
1800
2100
2400
2700
m/z
1420
1487
1462
1502
1583
1624
1649
1665
1794
1812
1816
1915
1940
1962
2058
2103
1956
1979
2125
1258
1340
1137
1096
1283
1299
1360
2247
2272
2291
2394
2416
2438

FIG. 10A (Cont.)

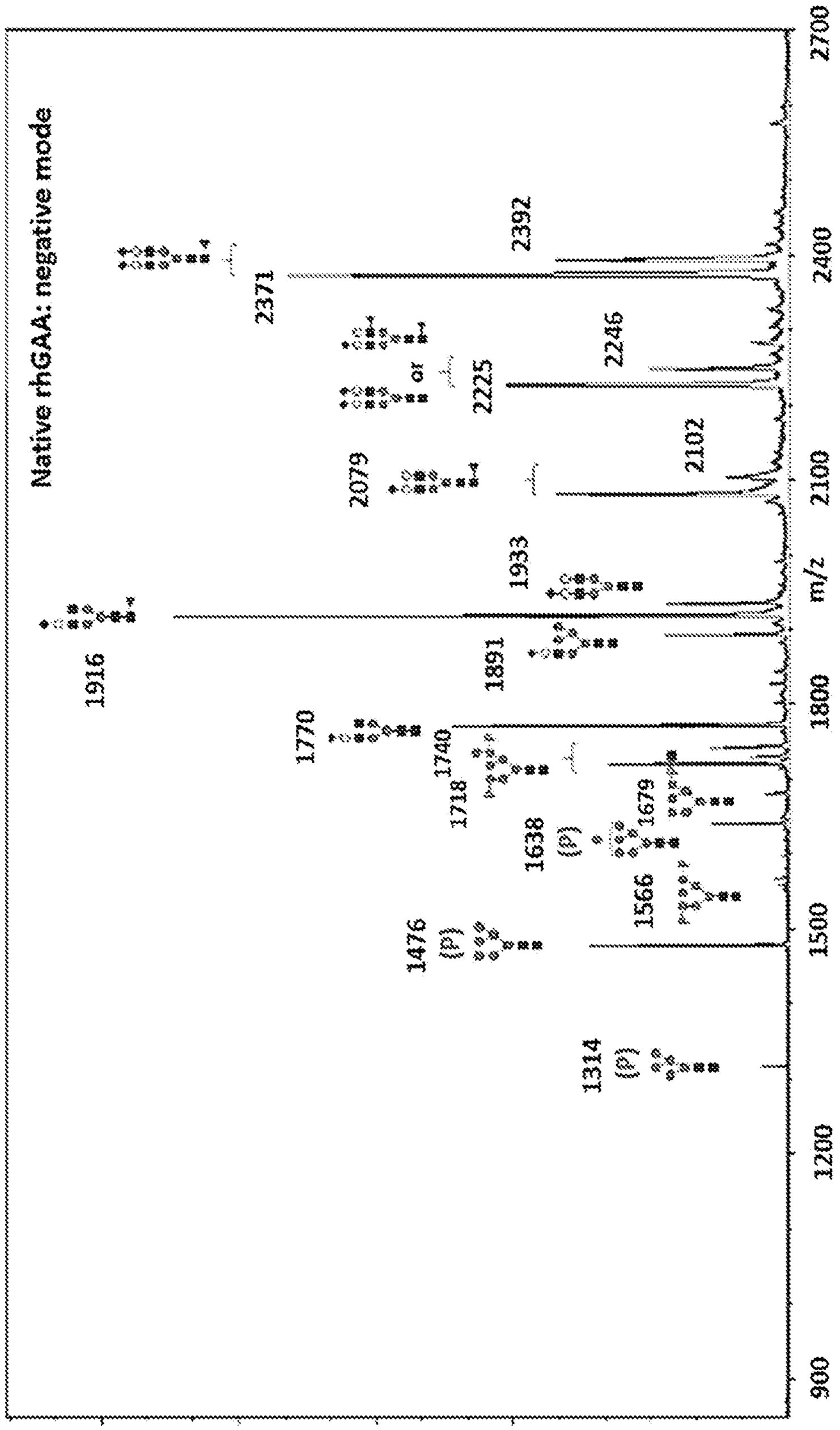

Native rhGAA: negative mode
2371
2392
2246
2225
2102
2079
1933
1916
1891
1770
1740
1718
1679
1638
1566
1476
1314
(P)
15000
12000
9000
6000
3000
900
1200
1500
1800
2100
2400
2700
m/z

FIG. 10B

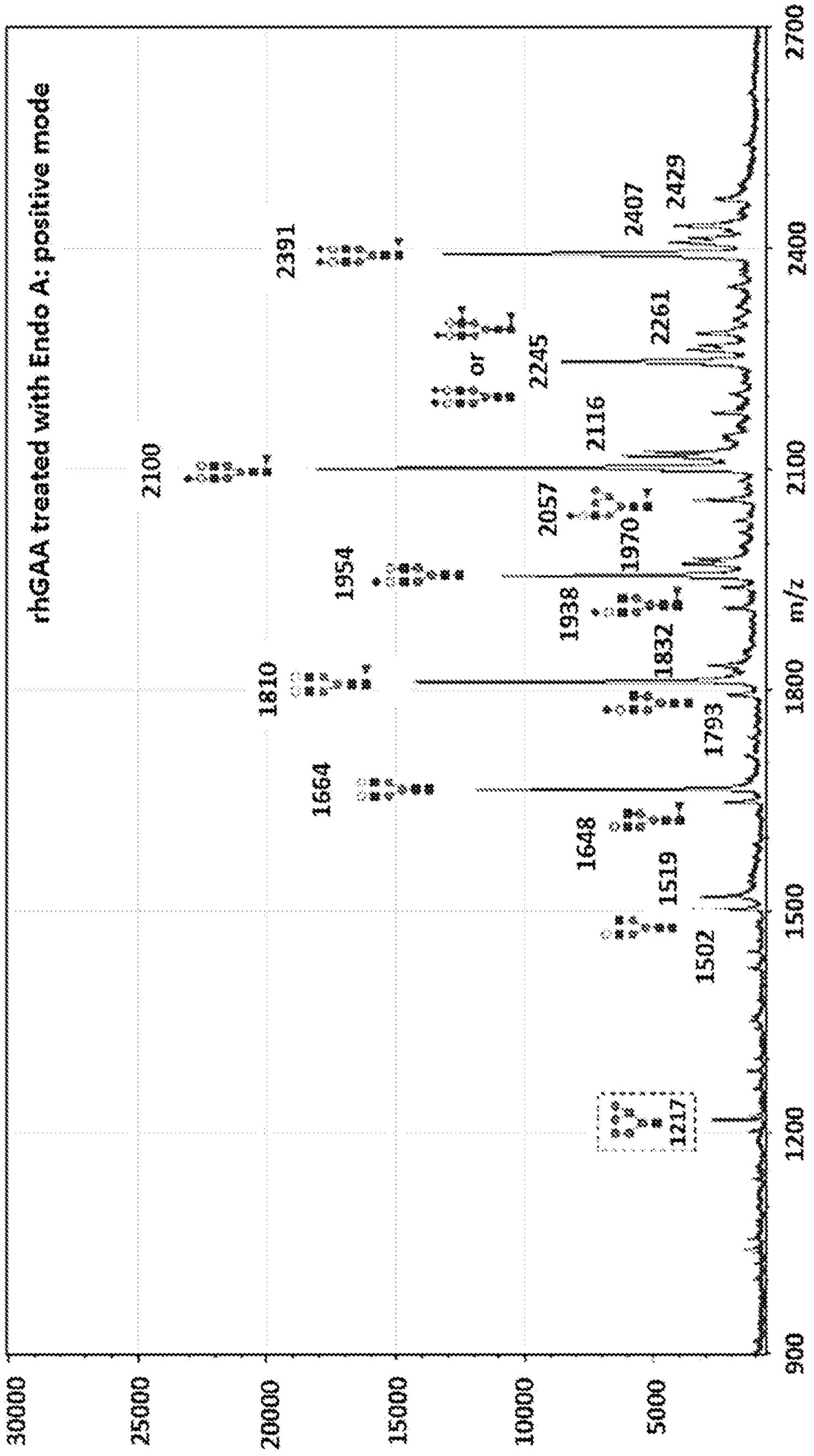

rhGAA treated with Endo A: positive mode
2391
2407
2429
2700
2400
2100
2057
2116
2245
2261
1938
1970
1954
1810
1832
1793
1664
1648
1519
1502
1217
30000
25000
20000
15000
10000
5000
1500
1800
2100
1200
900
m/z

FIG. 10B (Cont.)

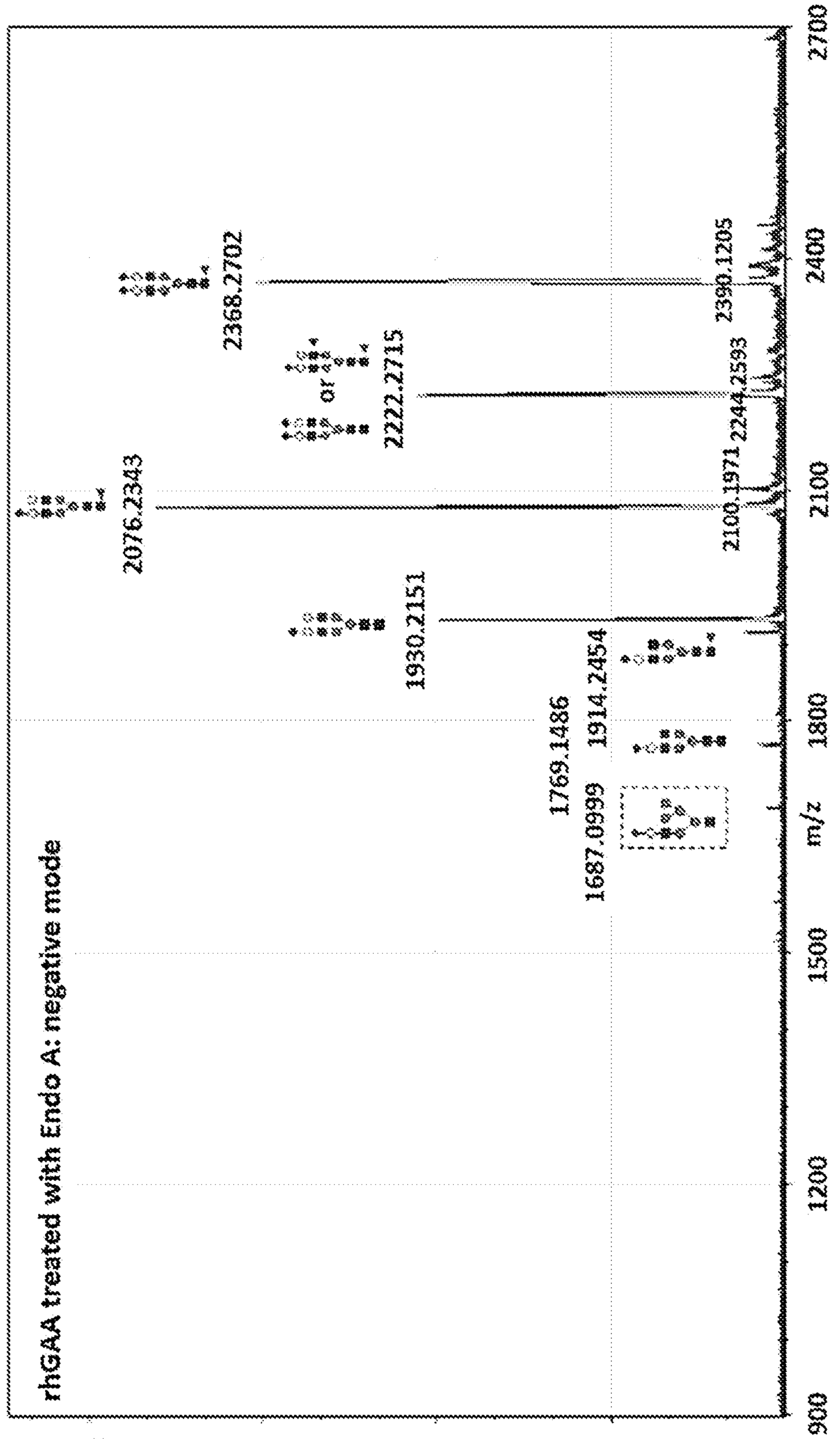
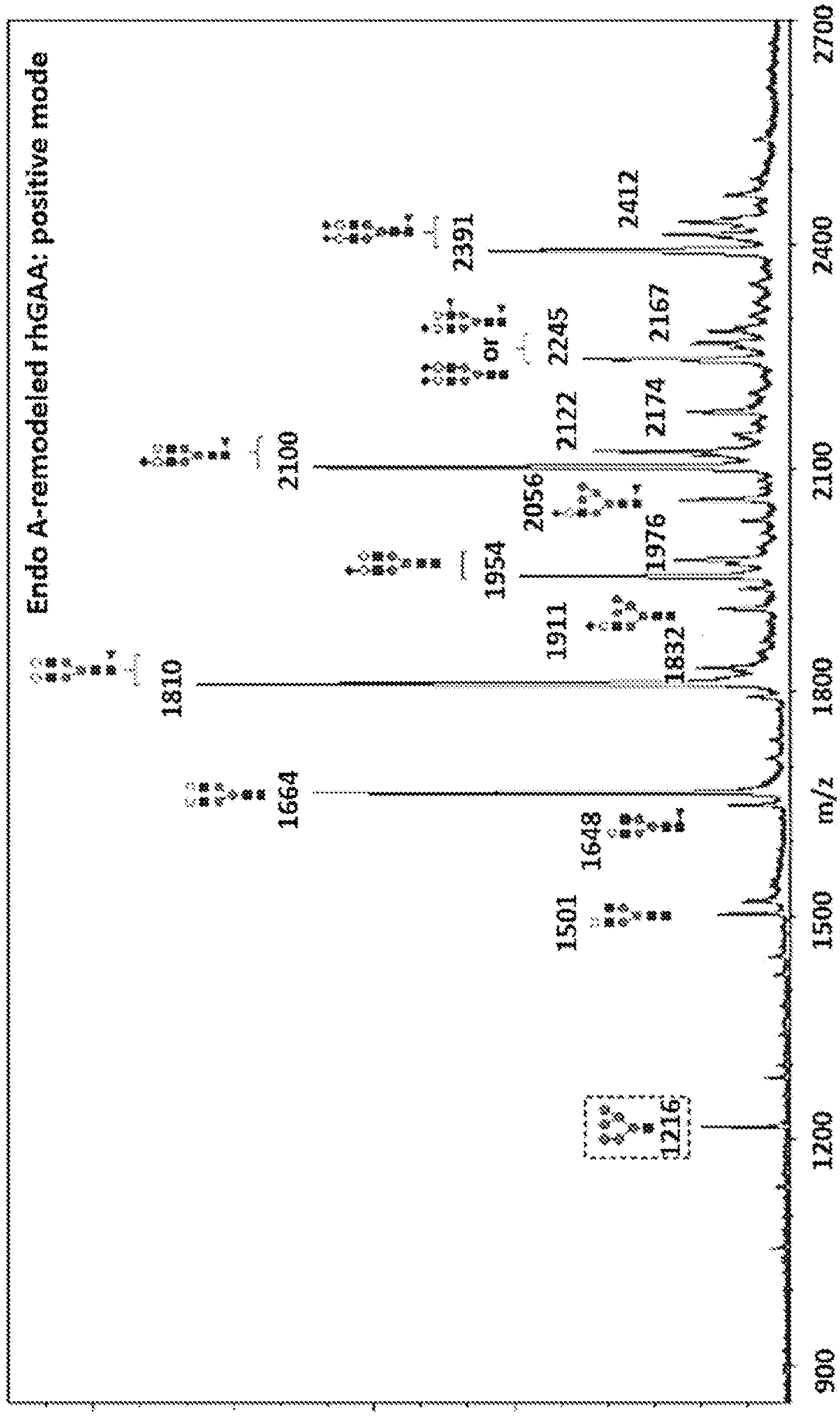

rhGAA treated with Endo A: negative mode
1200
900
600
300
900
1200
1500
1800
2100
2700
m/z
2076.2343
1930.2151
1769.1486
1687.0999
1914.2454
2222.2715
2100.1971
2244.2593
2390.1205
2368.2702
or

FIG. 10C


Endo A-remodeled rhGAA; positive mode
15000
12000
9000
6000
3000
m/z
900
1200
1500
1800
2100
2700
1216
1501
1648
1664
1810
1832
1911
1954
2056
2100
1976
2122
2174
2167
2245
2391
2412
2400

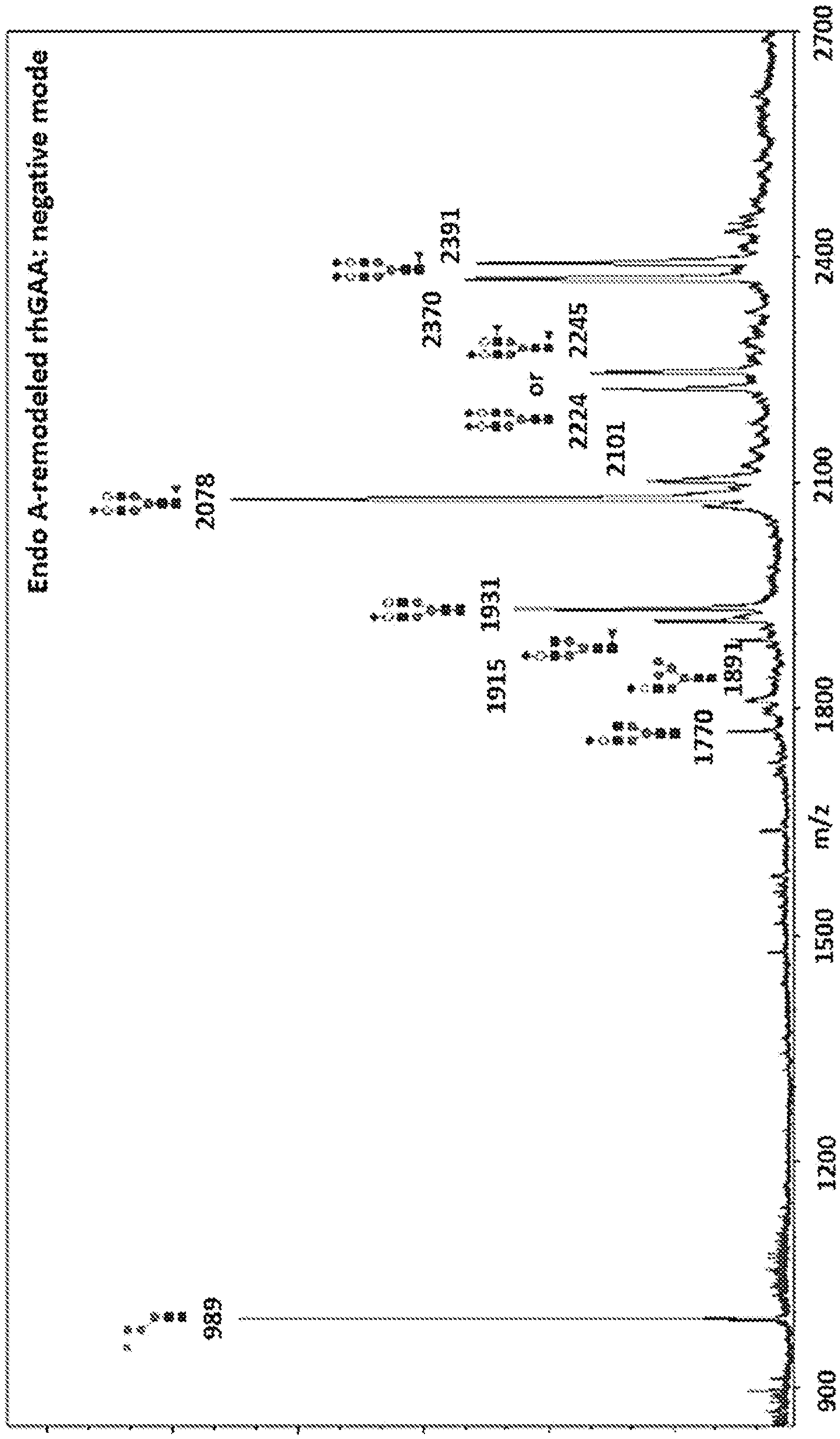

Endo A-remodeled rhGAA; negative mode
18000
15000
12000
9000
6000
3000
989
2078
1915
1931
1770
1891
2370
2391
2245
2224
2101
m/z
900
1200
1500
1800
2100
2400
2700

FIG. 10C (Cont.)

FIG. 10D

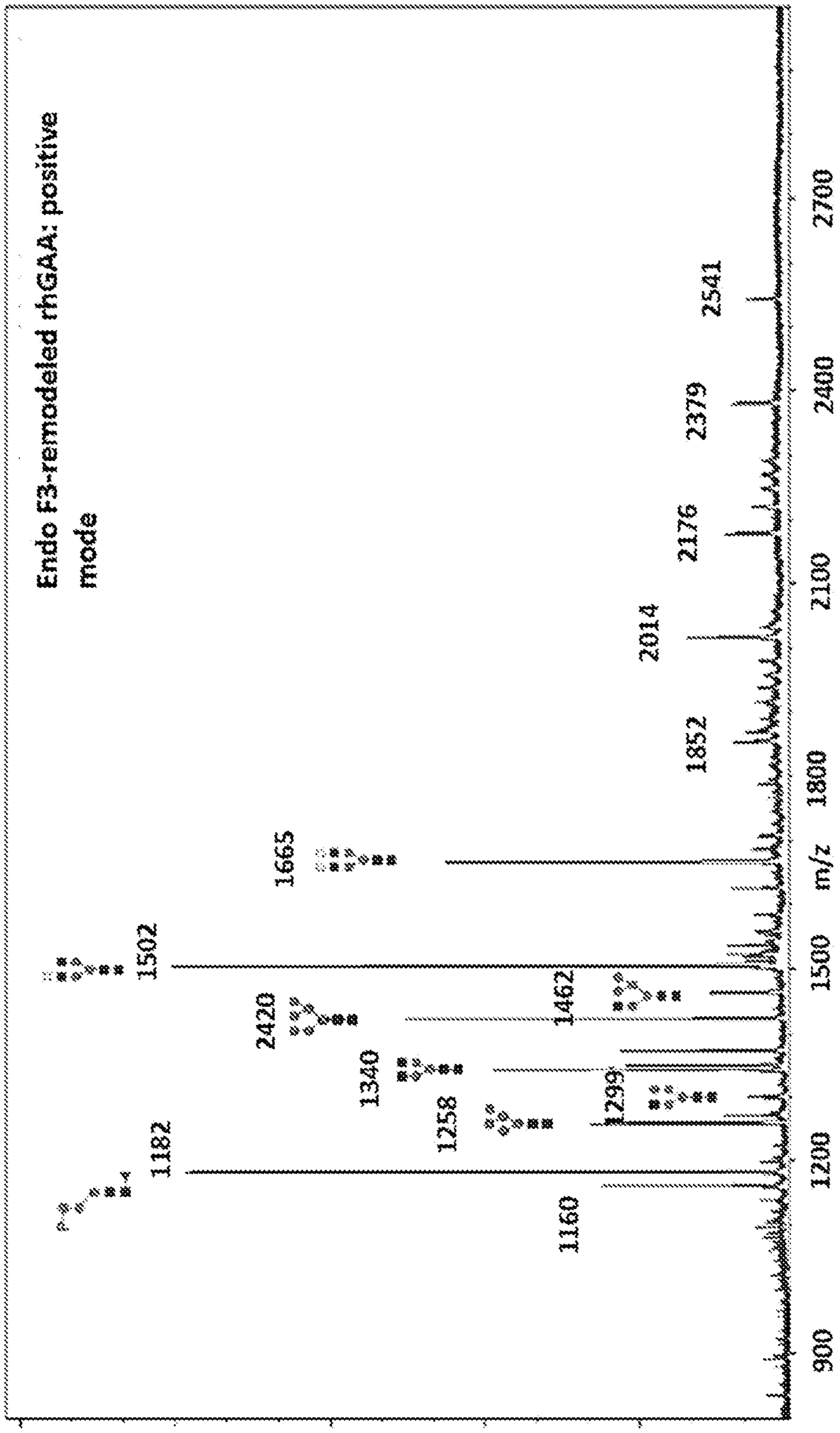
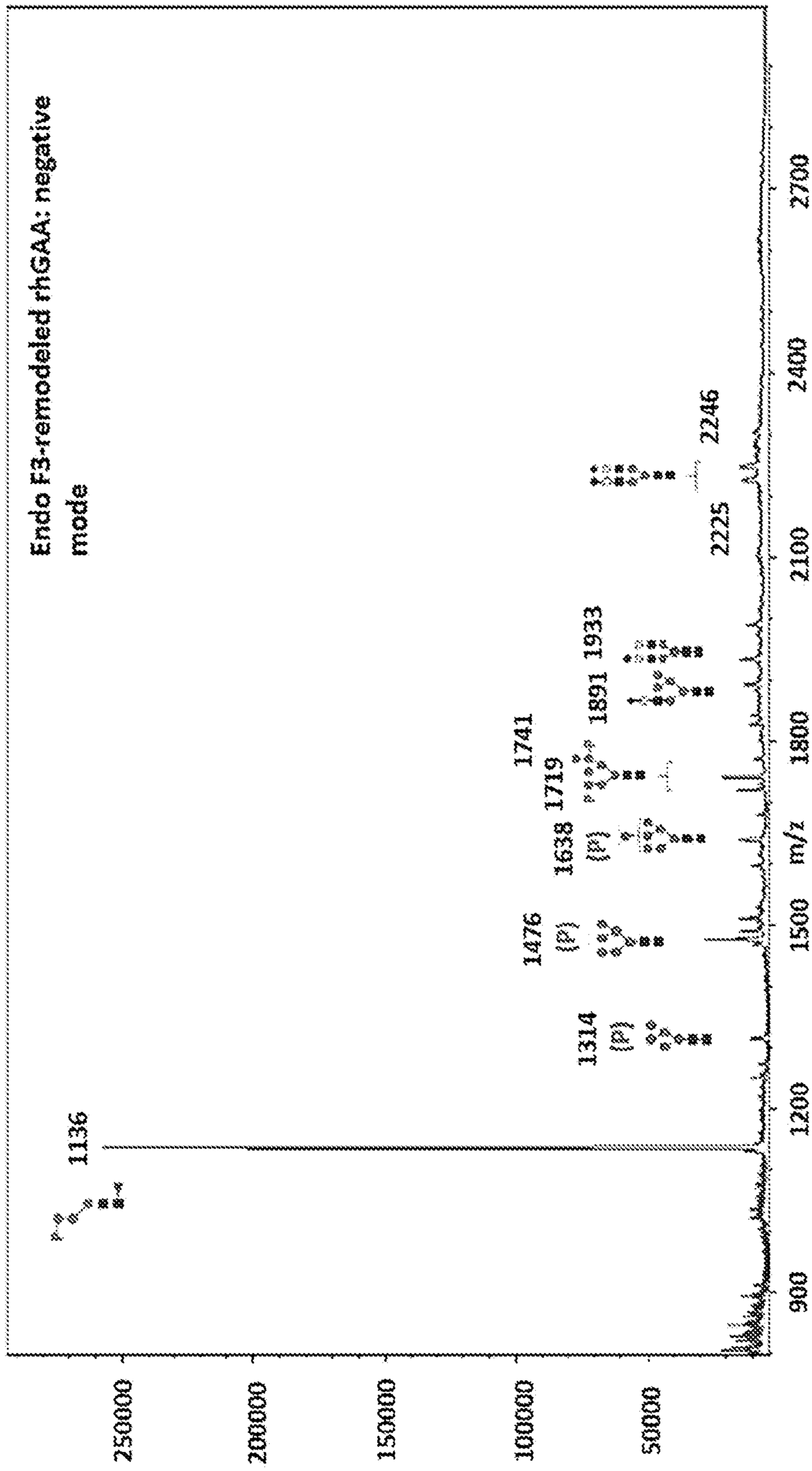

Endo F3-remodeled rhGAA: positive mode
15000
12000
9000
6000
3000
1182
1160
1258
1299
1340
1462
1502
1665
2014
1852
2176
2379
2420
2541
2700
900
1200
1500
1800
2100
2400
m/z

FIG. 10D (Cont.)


Endo F3-remodeled rhGAA: negative mode
2700
2400
2100
1800
1500
1200
900
2246
2225
1933
1891
1741
1719
1638
1476
1314
1136
250000
200000
150000
100000
50000
m/z

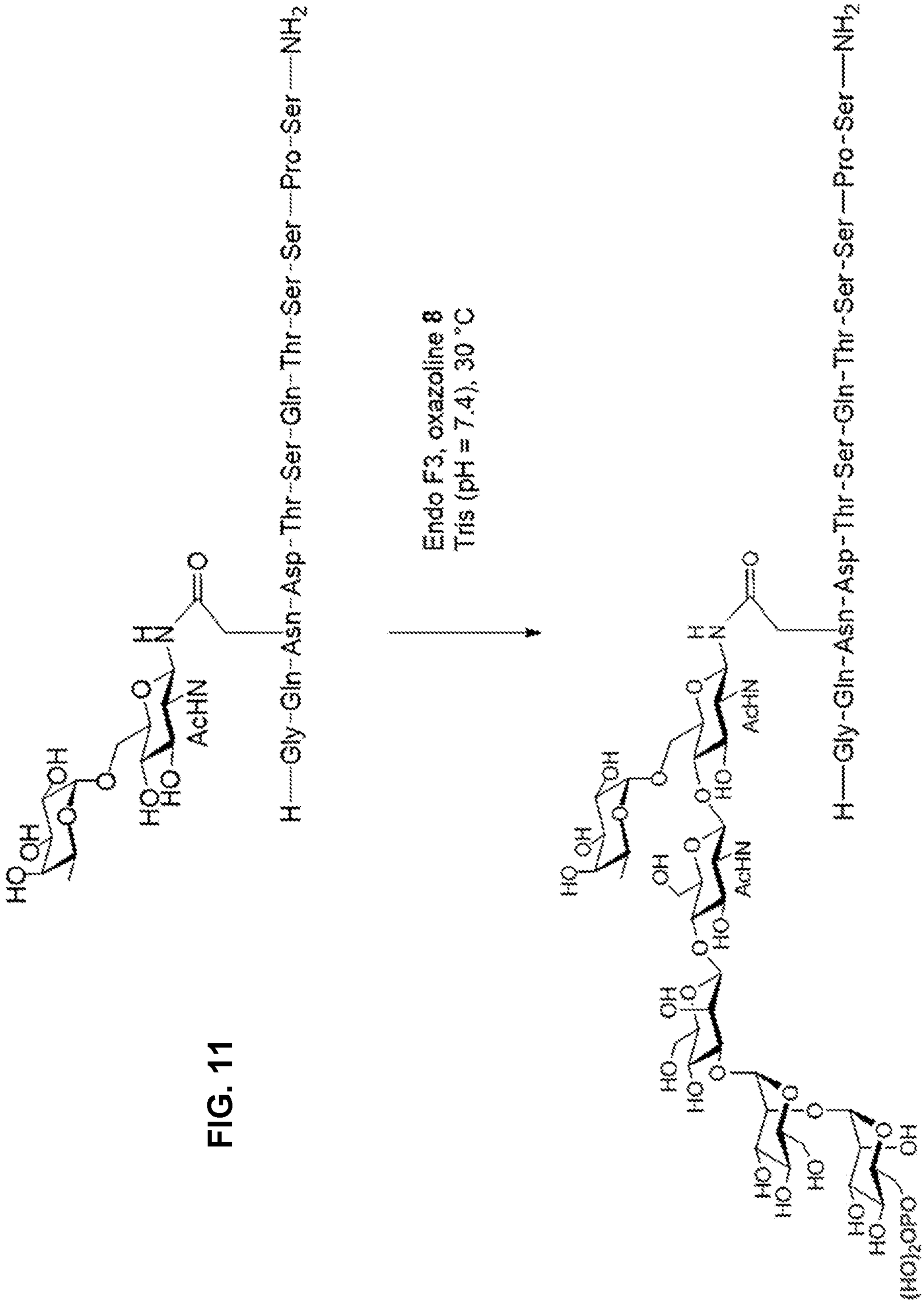
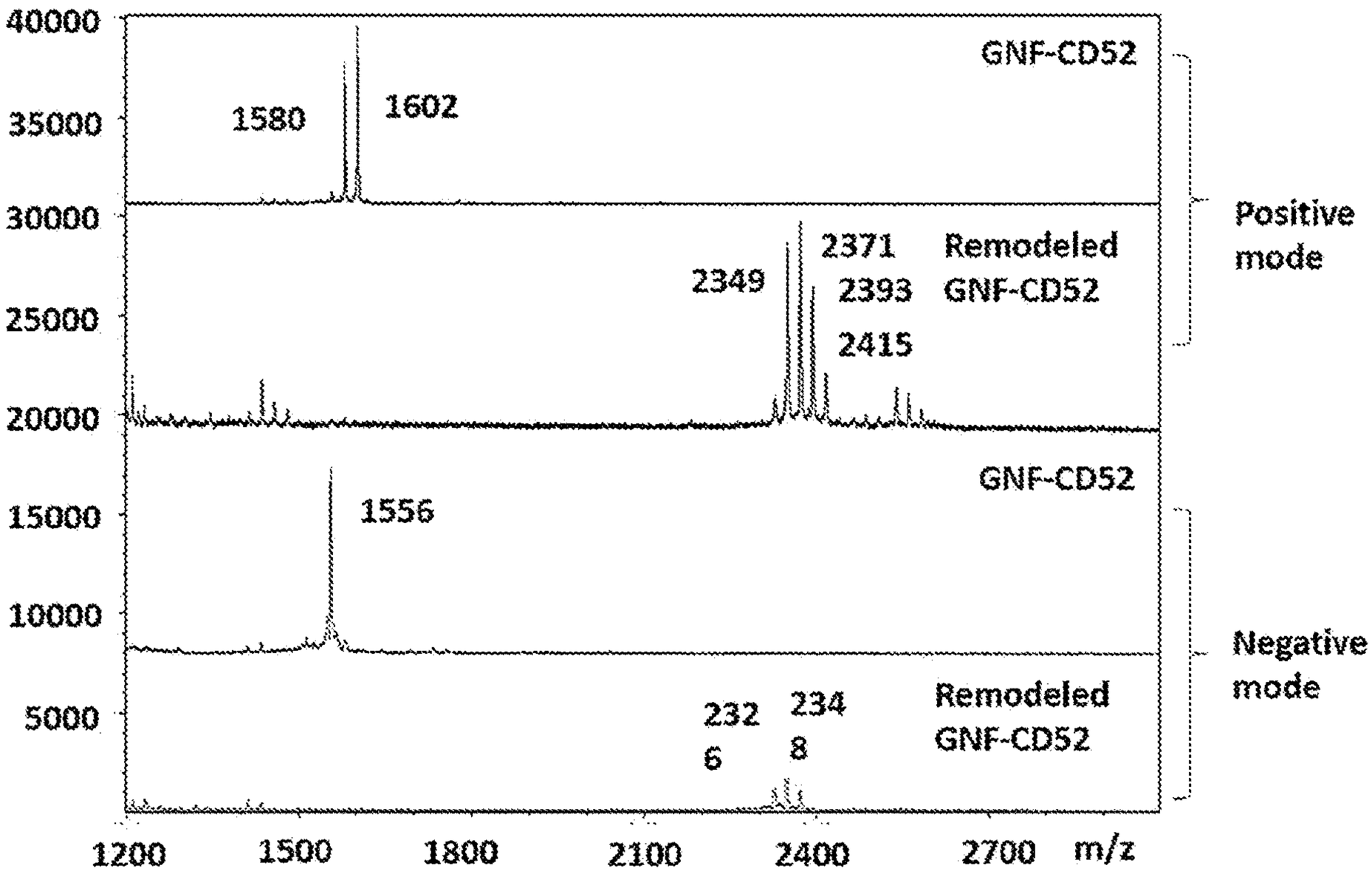

Endo F3, oxazoline 8
Tris (pH = 7.4), 30 °C
H—Gly-Gln-Asn-Asp-Thr-Ser-Gln-Thr-Ser—Pro-Ser—NH2
H—Gly-Gln-Asn-Asp-Thr-Ser-Gln-Thr-Ser—Pro-Ser—NH2
AcHN
HO
OH
HO
O
N
H

FIG. 11

FIG. 11 (Cont.)



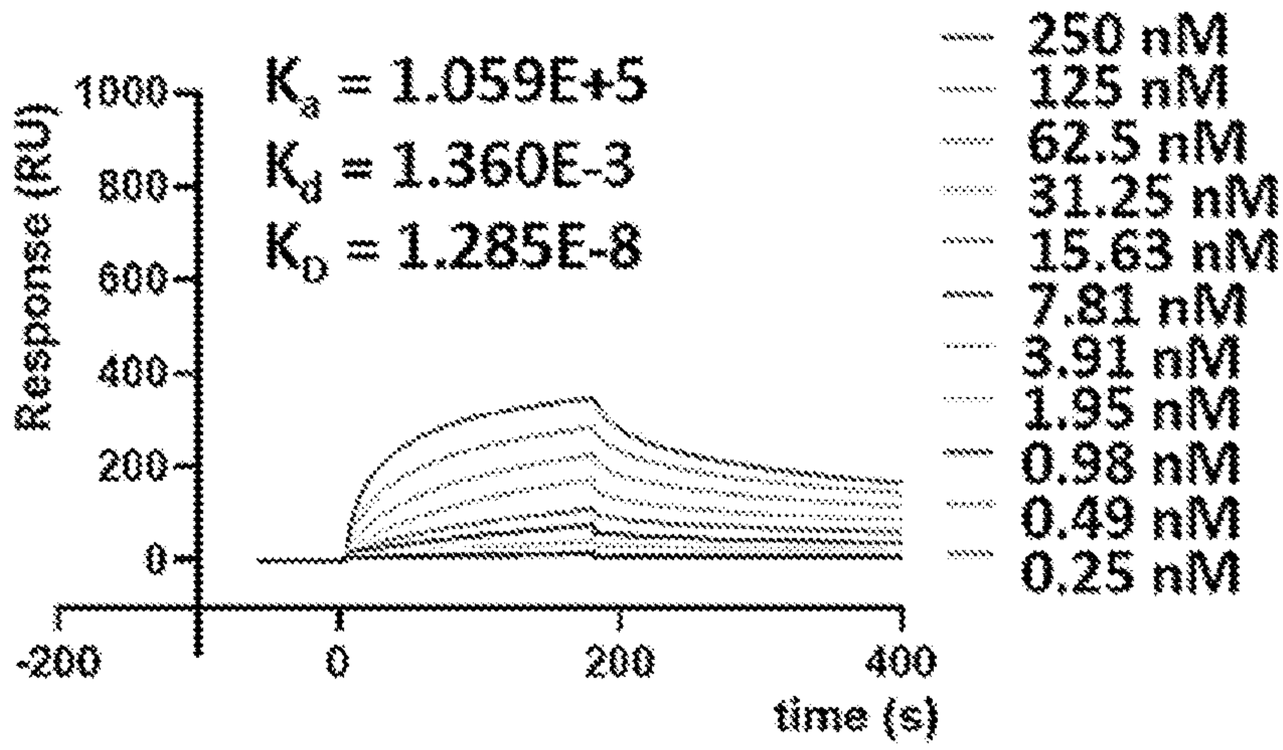
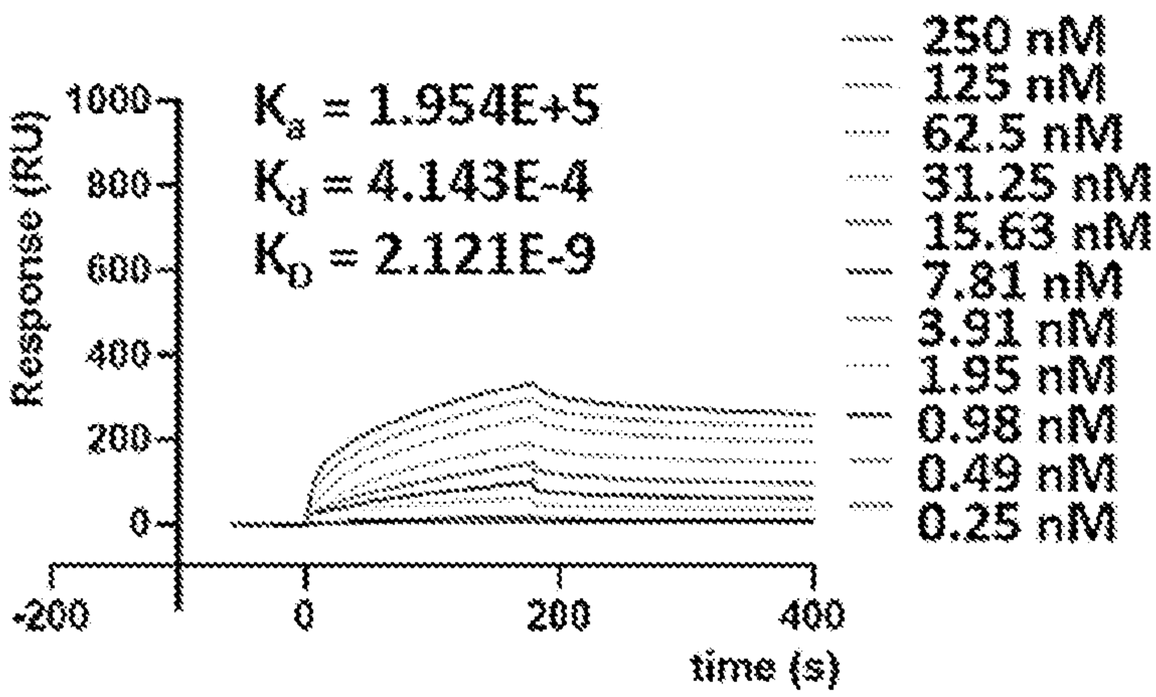
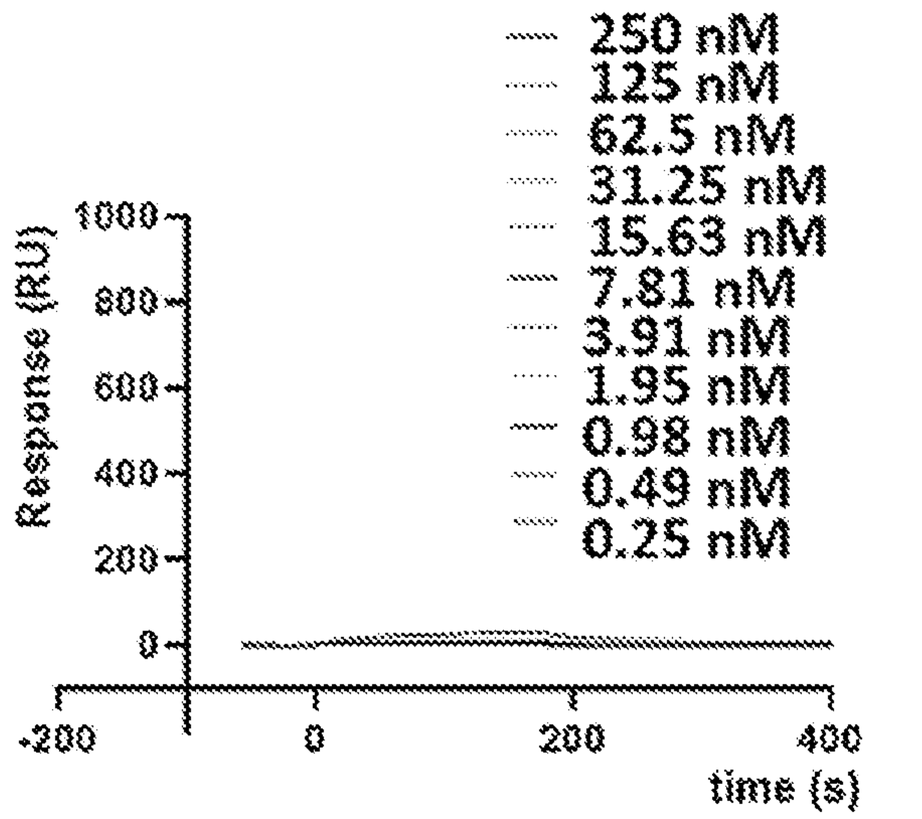


FIG. 12

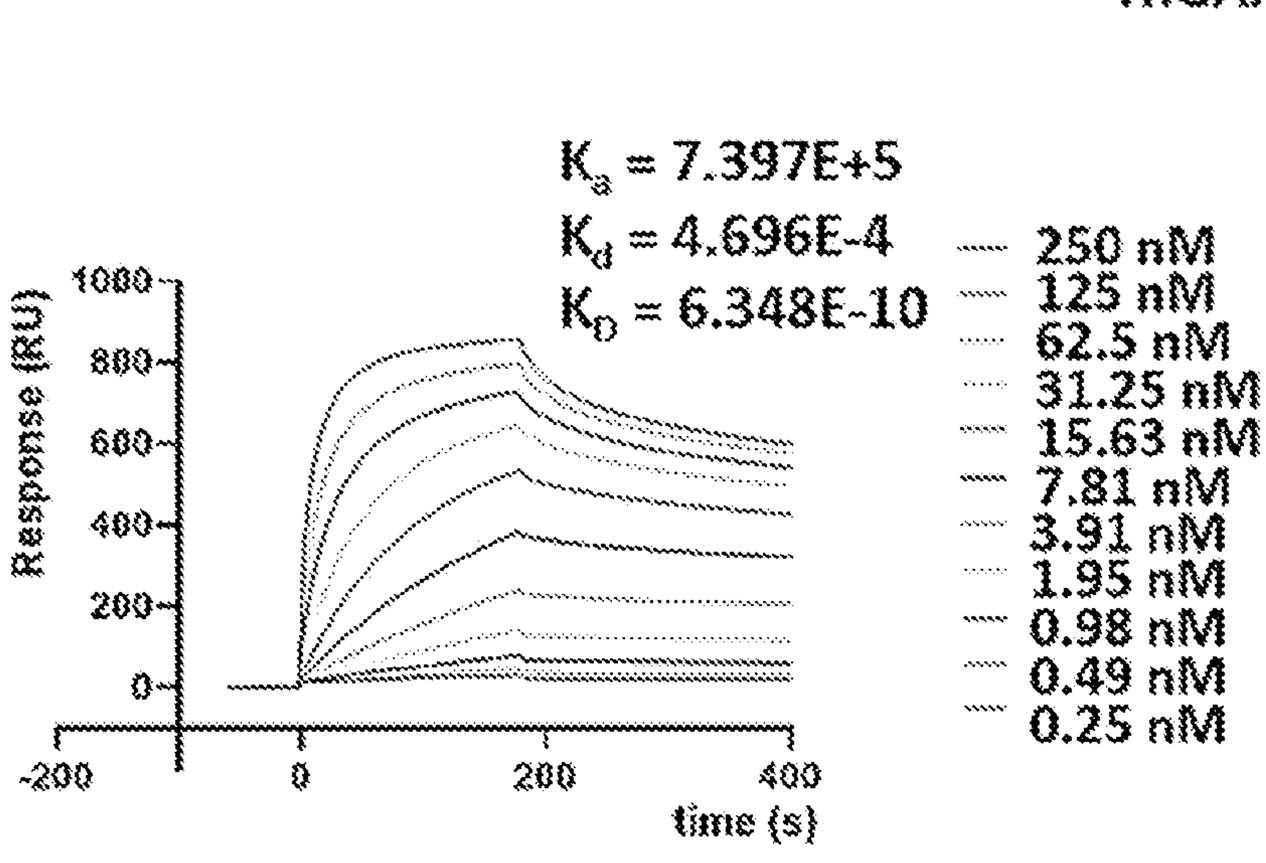


Endo A-remodeled rhGAA



rhGAA treated with Endo A

FIG. 12 (Cont.)



Endo F3-remodeled rhGAA

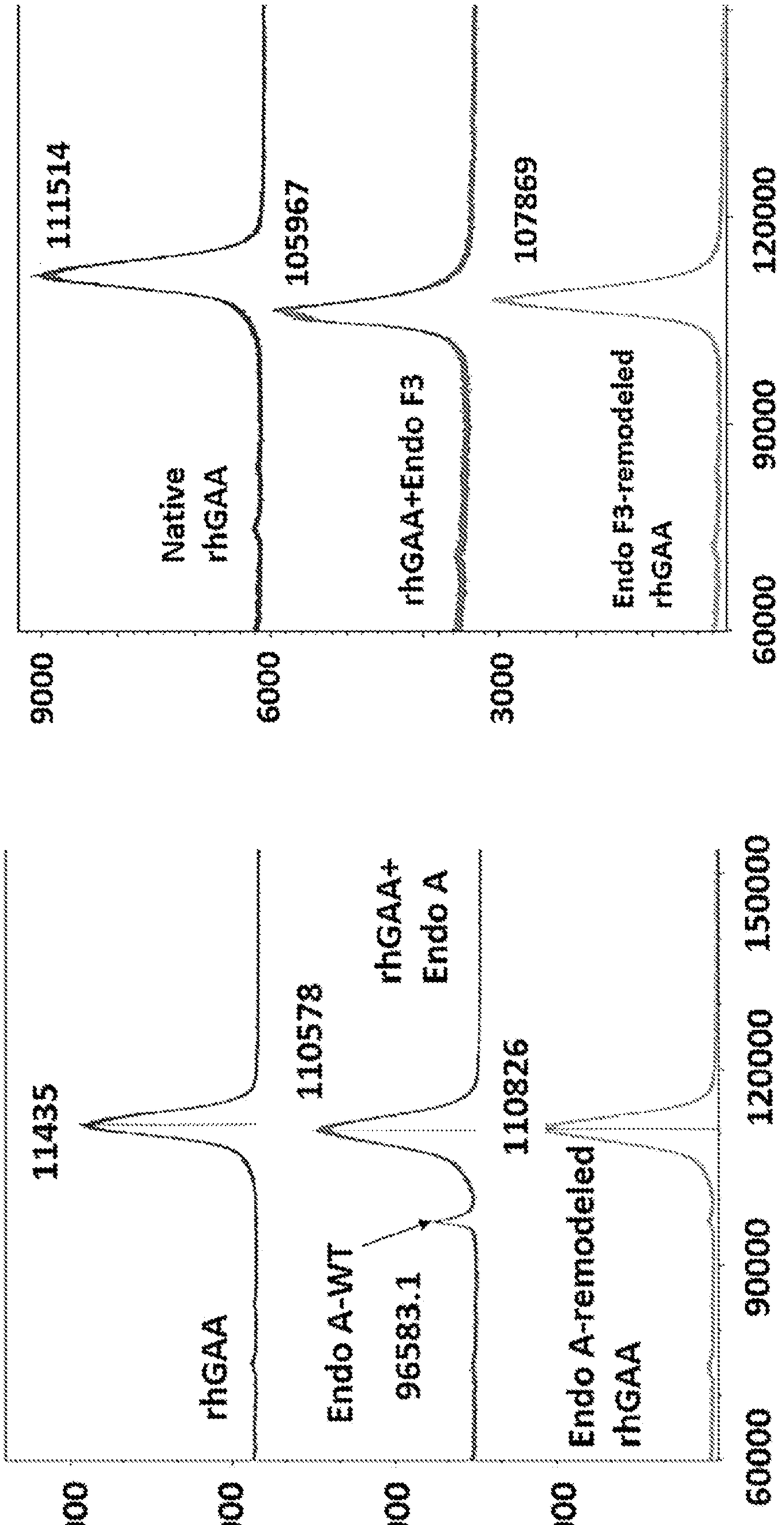

111514
Native rhGAA
105967
rhGAA+Endo F3
107869
Endo F3-remodeled rhGAA
120000
9000
6000
3000
60000
90000
11435
rhGAA
110578
Endo A-WT
96583.1
110826
Endo A-remodeled rhGAA
rhGAA+ Endo A
12000
150000
120000

FIG. 13

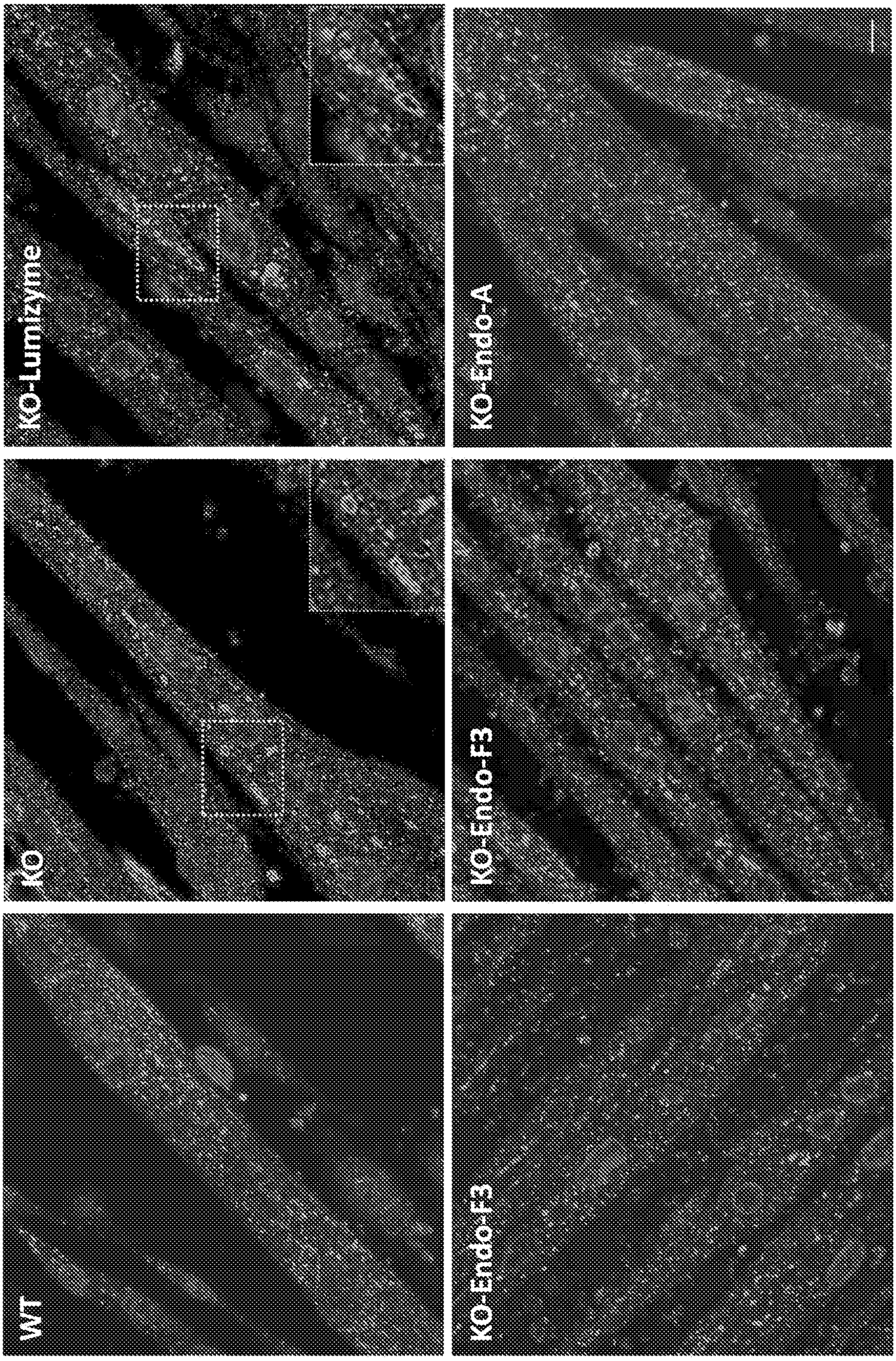


FIG. 14

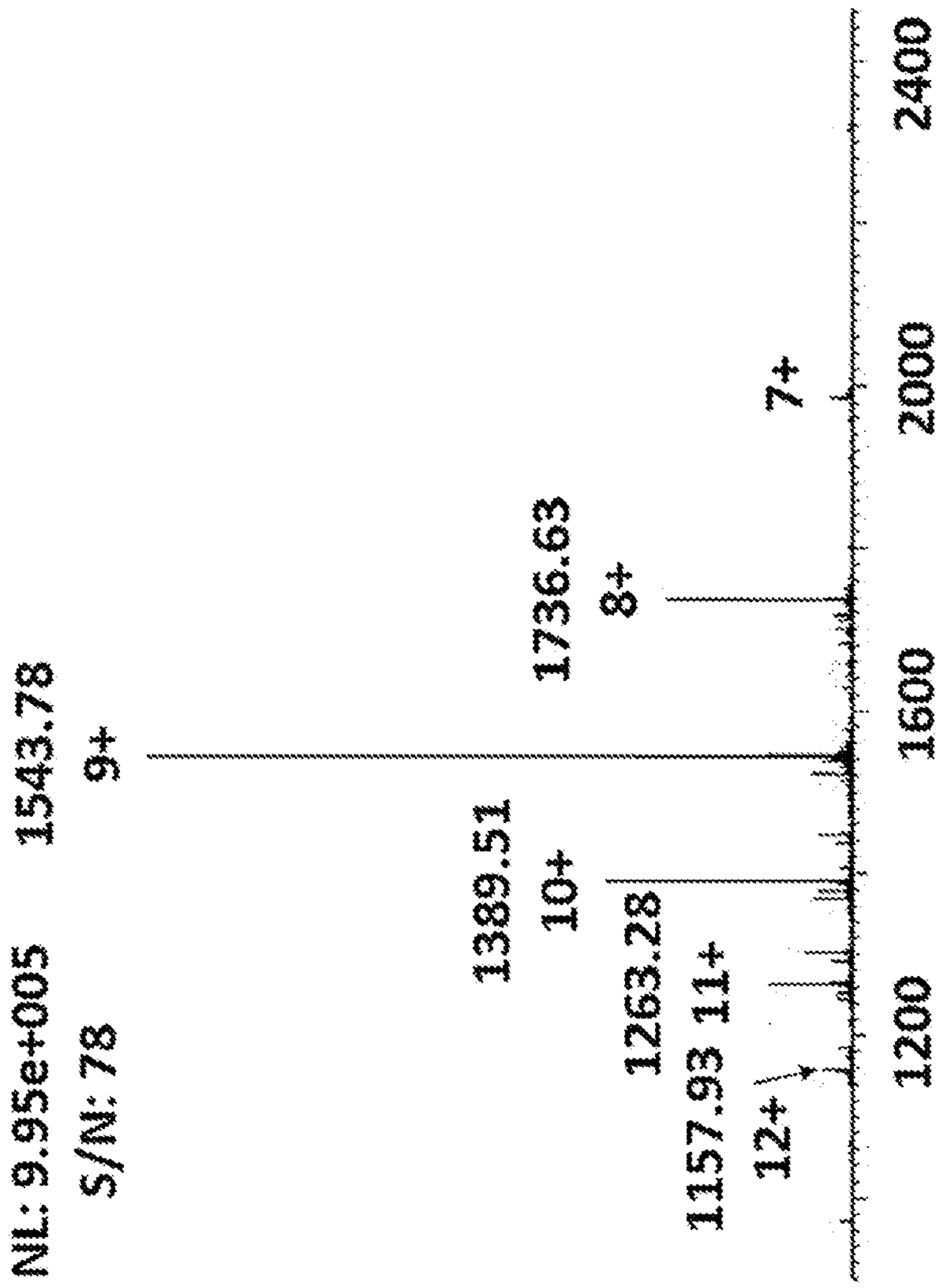

2400
NL: 9.95e+005
S/N: 78
1543.78
9+
1736.63
8+
1389.51
10+
1263.28
11+
1157.93
12+
7+
1200
1600
2000

FIG. 15

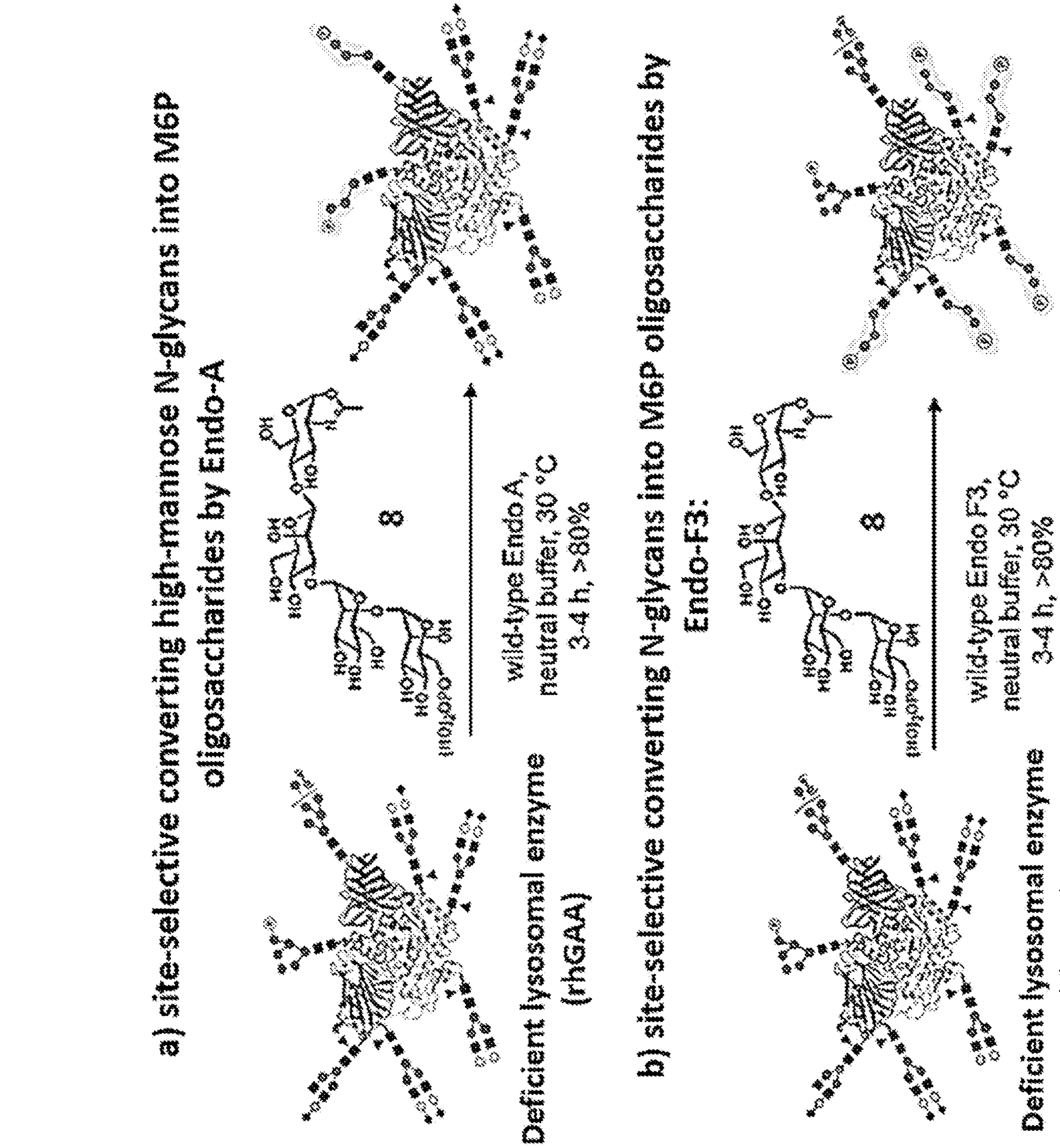
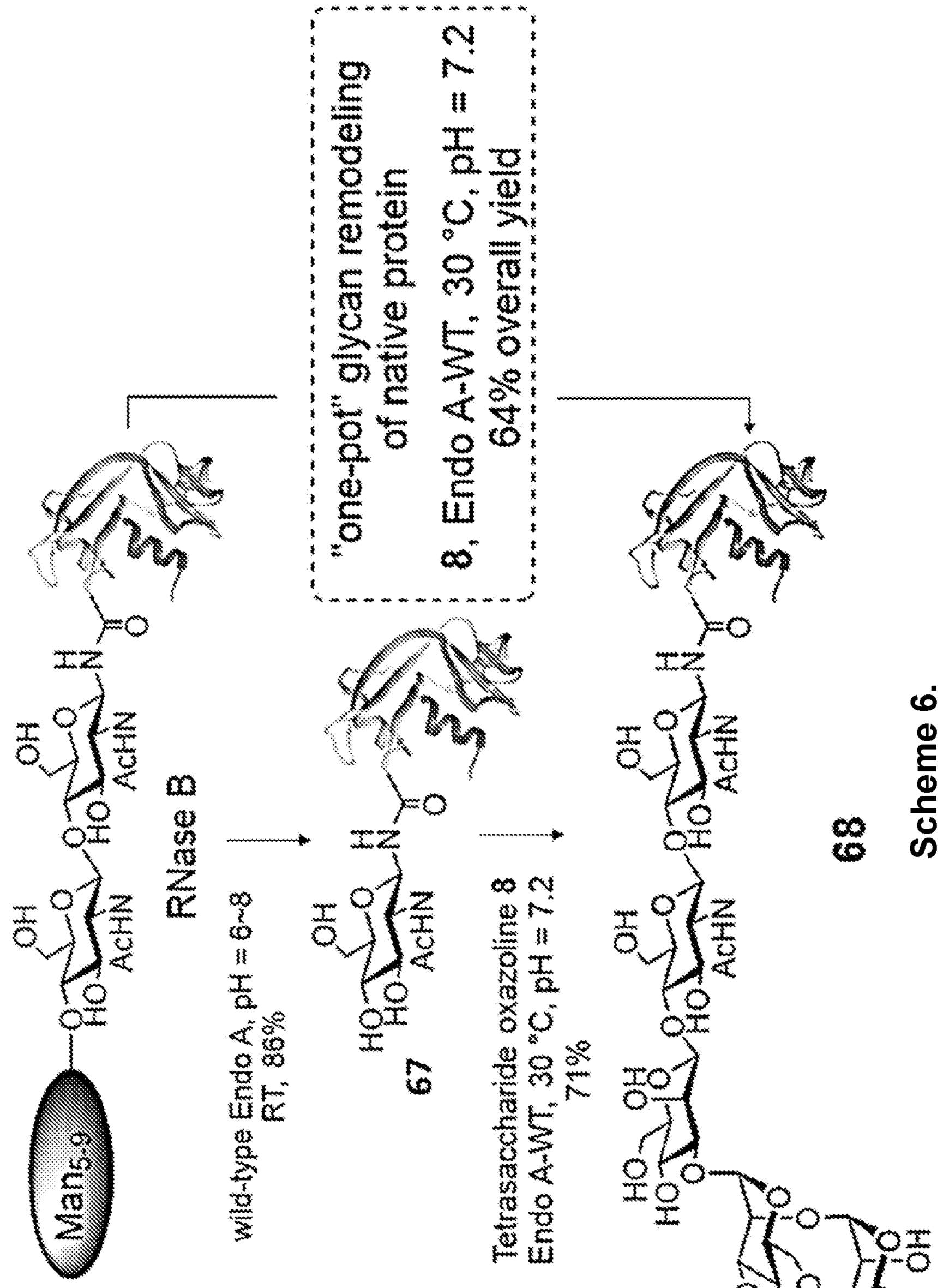
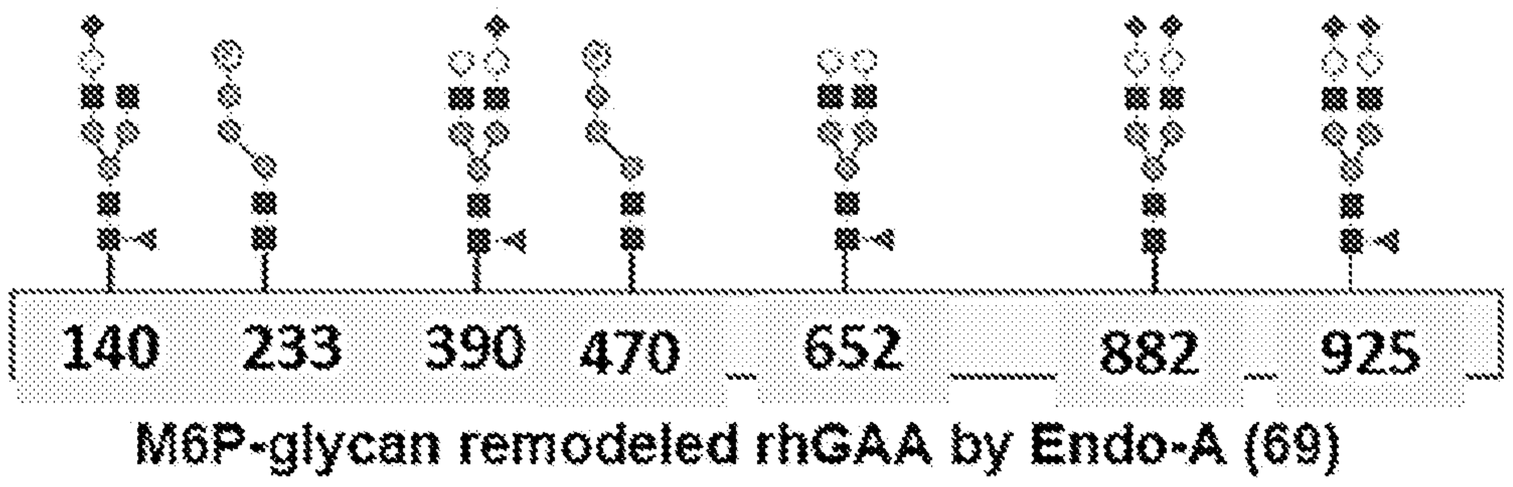

a) site-selective converting high-mannose N-glycans into M6P oligosaccharides by Endo-A
8
wild-type Endo A,
neutral buffer, 30 °C
3-4 h, >80%
Deficient lysosomal enzyme
(rhGAA)
b) site-selective converting N-glycans into M6P oligosaccharides by Endo-F3:
8
Wild-type Endo F3,
neutral buffer, 30 °C
3-4 h, >80%
Deficient lysosomal enzyme
(rhGAA)

FIG. 16



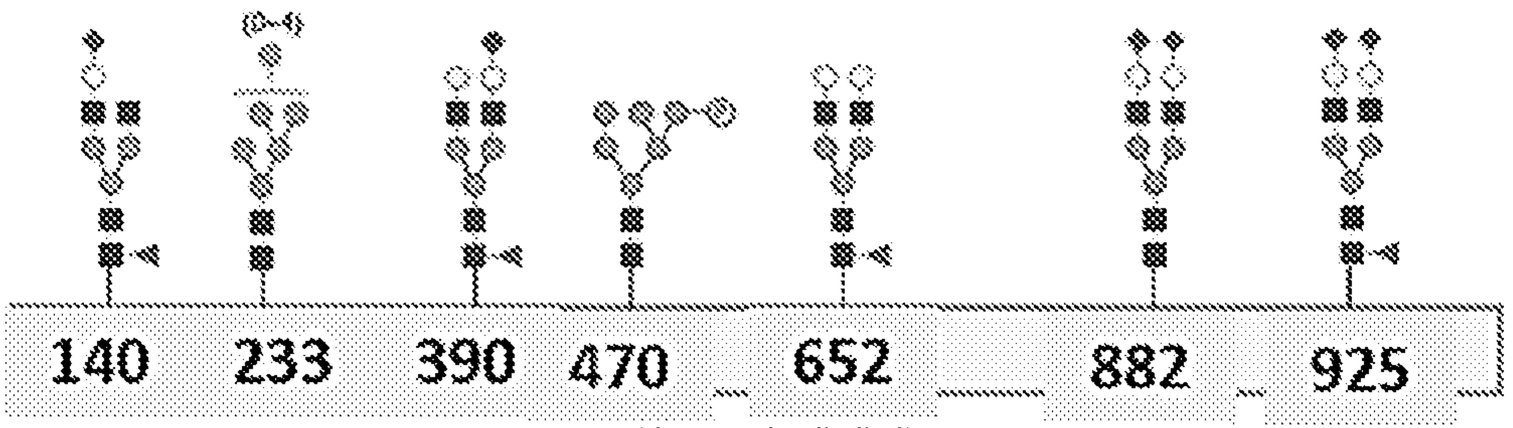
Scheme 6.

FIG. 17



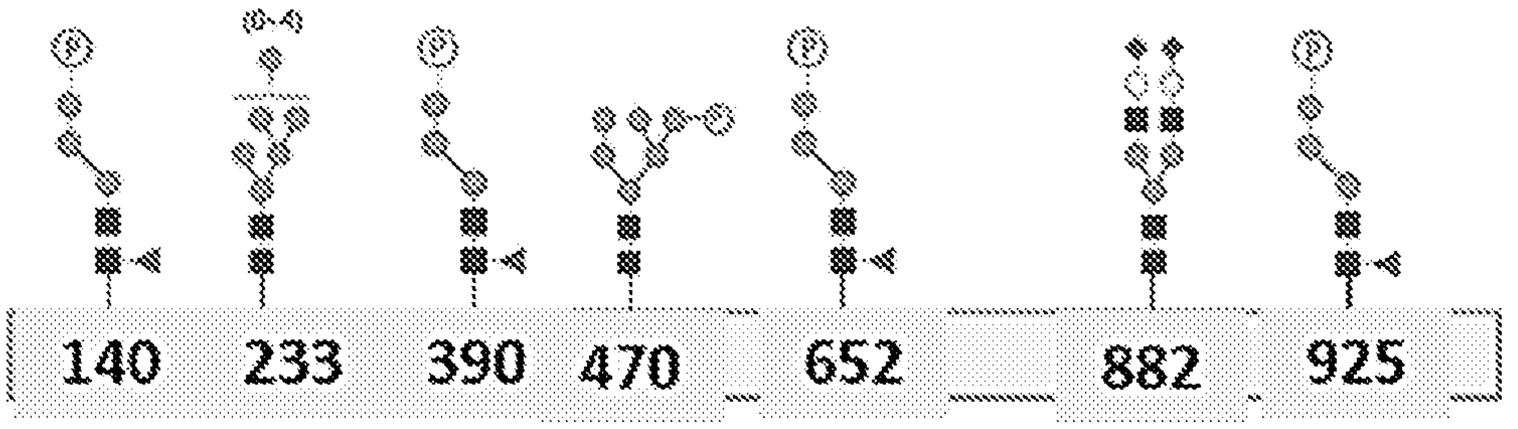
M6P-glycan remodeled rhGAA by Endo-A (69)

Endo A-WT, oxazoline **8**
PBS (pH = 7.2), 30 °C, 86%



native rhGAA

Endo F3-WT, oxazoline **8**
PBS (pH = 7.2), 30 °C, 71%



M6P-glycan remodeled rhGAA by Endo-F3 (70)

Scheme 7

FIG. 18

SITE-SPECIFIC GLYCAN REMODELING OF LYSOSOMAL ENZYMES AND APPLICATIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/178,731, filed on Apr. 23, 2021 and U.S. Provisional Application No. 63/264,011, filed on Nov. 12, 2021, the contents of which hereby are incorporated herein by reference each in their respective entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant Number R01GM080374E awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

SEQUENCE LISTING

[0003] A Sequence Listing accompanies this application and is submitted as an ASCII text file of the sequence listing named "171351_00039_ST25.txt" which is 140,992 bytes in size and was created on Apr. 25, 2022. The sequence listing is electronically submitted via EFS-Web with the application and is incorporated herein by reference in its entirety.

BACKGROUND

[0004] Many diseases are associated with the altered or reduced expression of a protein, enzyme, or metabolite. For example, lysosomal storage diseases (LSDs) are a group of inherited metabolic disorders caused by deficiency of respective hydrolases that are responsible for the degradation of substrates stored in lysosomes.[1] The accumulation of undigested macromolecules leads to cell dysfunction and progressive clinical manifestations.[2] A variety of therapeutic approaches have been attempted for LSDs,[3] with intravenous enzyme replacement therapy (ERT) being the most prevalent.[4] However, the effectiveness of ERT varies among different LSDs. For example, in the case of Pompe disease that is caused by a deficiency of the lysosomal glycogen-degrading enzyme, the acid α-glucosidase (GAA), a very high dose of the recombinant human acid α-glucosidase (rhGAA, Lumizyme) needs to be administered due to its relatively low cellular uptake and poor drug targeting.[5] The cation-independent mannose-6-phosphate (M6P) receptor (CI-MPR), which continuously traffics between plasma membrane, late endosomes and Trans-Golgi network (TGN), plays a critical role in cellular uptake and intracellular transport of enzymes to lysosomes by recognizing the M6P-containing N-glycans attached to the enzymes.[6-9] Thus, enhancement of the CI-MPR-mediated endocytosis represents a major strategy to improve the overall efficiency of the ERT-based treatments.[10-12] Toward this end, several approaches for increasing M6P modification of lysosomal enzymes have been attempted, including chemical conjugation of synthetic M6P-containing glycans,[13-22] construction of non-mammalian based platforms to improve the M6P content,[23-26] gene engineering of the glycosylation pathways,[27, 28] and a chemoenzymatic remodeling approach to introduce synthetic phosphorylated N-glycans.[29, 30] There are examples that the resulting modified enzymes show increased binding to CI-MPR and enhanced uptake by cells compared with the unmodified enzymes. Despite these promising studies, however, these approaches can be tedious and difficult to control, resulting in mixtures of different glycoforms with varied stability and biological activities or with side effects.

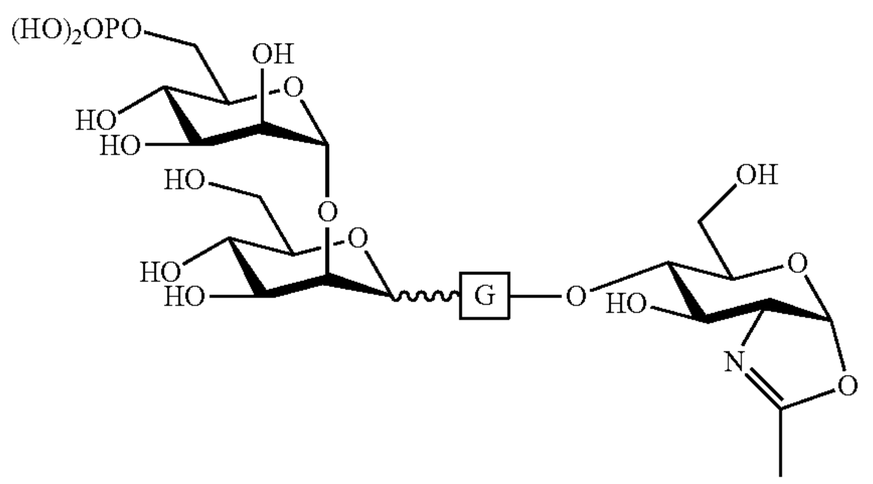
[0005] Thus, there remains an unmet need to identify the essential structures of M6P-containing N-glycans that exhibit high affinity for the CI-MPR and to develop site-selective conjugation of the M6P glycan ligands to be able to deliver a therapeutic agent, for example, therapeutic lysosomal enzymes to achieve structurally well-defined products.

SUMMARY

[0006] The present disclosure relates to phosphorylated N-glycan compounds that can be used for one-pot glycan remodeling of glycoproteins, in particular therapeutic lysosomal enzymes, to provide enhanced receptor binding, cellular uptake, and overall therapeutic efficacy.

[0007] In one aspect, the disclosure provides a compound of Formula (I), or a salt thereof,

(I)



[0008] wherein G is sugar moiety or linker.

[0009] In another aspect, the disclosure provides a method for remodeling a glycoprotein. The method comprises (a) contacting the glycoprotein with an endoglycosidase selected from the group consisting of wild type Endo A, wild type Endo F3, wild type Endo-CC, and a combination of, thereby producing a deglycosylated intermediate comprising a N-acetylglucosamine (GlcNAc) or core-fucosylated N-acetylglucosamine (Fuca1,6GlcNAc) acceptor from the glycoprotein by a deglycosylation activity of the endoglycosidase to produce a deglycosylated intermediate; and (b) contacting a glycan oxazoline comprising a mannose-6-phosphate (M6P) moiety with the deglycosylated intermediate in the presence of the endoglycosidase, thereby attaching the glycan oxazoline to the N-acetylglucosamine (GlcNAc) or core-fucosylated N-acetylglucosamine (Fuca1, 6GlcNAc) acceptor by a transglycosylation activity of the endoglycosidase, thereby producing a remodeled glycoprotein, wherein (a) and (b) are carried out in a one-pot reaction.

[0010] In yet another aspect, the disclosure provides a method of enhancing binding affinity of a glycoprotein to a cation-independent M6P receptor (CI-MPR), comprising remodeling the glycoprotein according to the method described herein and contacting the glycoprotein with a cell comprising CI-MPR receptor, thereby enhancing binding affinity of the glycoprotein to the CI-MPR.

[0011] In another aspect, the disclosure provides a method of enhancing or increasing uptake of a glycoprotein in a cell. The method comprises (a) remodeling the glycoprotein according to the method described herein, and (b) contacting the cell with the remodeled glycoprotein, thereby enhancing uptake of the glycoprotein in the cell.

[0012] In a further aspect, the disclosure provides a glycan-remodeled glycoprotein produced by the method described herein. In some aspect, the glycan-remodeled glycoprotein is a glycan-remodeled lysosomal enzyme.

[0013] In yet a further aspect, the disclosure provides a method of treating Pompe disease in a subject in need thereof. The method comprises administering to the subject a pharmaceutically effective amount of the glycan-remodeled lysosomal enzyme described herein.

[0014] The foregoing and other aspects and advantages of the embodiments of the present disclosure will appear in from the following description. In the description, reference is made to the accompanying drawings which form a part hereof, and in which there is shown by way of illustration preferred embodiments of the disclosure. Such embodiments are illustrative only, are not intended to be limited, and do not necessarily represent the full scope of the present disclosure, however, and reference is made therefore to the claims herein for interpreting the scope of the teachings of the present disclosure. As such, features of the presently disclosed subject matter will be apparent from the following detailed description and the appended claims that follow.

List of Abbreviations

[0015] The following list of abbreviations are used in the understanding of the present disclosure: lysosomal storage disease (LSD), enzyme replacement therapy (ERT), acid α-glucosidase (GAA), recombinant human acid α-glucosidase (rhGAA), mannose (Man), mannose-6-phosphate (M6P), cation-independent mannose-6-phosphate receptor (CI-MPR), Trans-Golgi network (TGN), High Performance Liquid Chromatography (HPLC), Electrospray Ionization Mass Spectrometry (ESI-MS).

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0017] FIG. **1** Structures of synthetic M6P-containing N-glycan oxazolines used for structure-activity relationship studies of enzymatic transglycosylation and M6P receptor binding.

[0018] FIGS. **2**A-**2**F Glycan analysis (negative mode) and representative sensorgrams of three independent SPR binding experiments of native rhGAA and M6P glycan-remodeled rhGAA (69 and 70). Average K_D values for native rhGAA, 69 and 70 were 14.0±3.7 nM, 2.3±0.2 nM and 0.63±0.07 nM, respectively.

[0019] FIG. **3** Comparison of the enzyme activity of commercial rhGAA and the M6P glycan-remodeled rhGAA (69 and 70).

[0020] FIGS. **4**A-**4**D show the effect of the therapeutic enzymes in GAA KO (KO) myotubes. FIG. **4**A shows that GAA activity was measured in cell lysates from untreated-(KO) and treated KO myotubes following incubation with Lumizyme (KO-Lumizyme), Endo-F3 remodeled rhGAA (KO-Endo-F3), and Endo-A remodeled rhGAA (KO-Endo-A). GAA activity in cell lysates of wild type (WT) myotubes was measured for comparison. The KO cells were treated for 24 hours with 5 μM of each of the recombinant enzymes; n=5 for each condition. Statistical significance was determined by one-way ANOVA; the graph represents mean+SD. * $p<0.05$; **** $p<0.0001$; ns—statistically insignificant. FIG. **4**B shows Western blot analysis (with anti-human GAA antibodies) of muscle cell lysates treated with the recombinant enzymes; GAPDH was used as a loading control. FIG. **4**C shows Western blot analysis of the recombinant enzymes with anti-human GAA antibodies. FIG. **4**D shows Western blot analysis of WT, untreated- and treated muscle cell lysates with lysosomal (anti-LAMP1) and endosomal (anti-Rab5) antibodies. A decrease in the levels of both markers is observed in Endo-F3- and Endo-A treated cells.

[0021] FIGS. **5**A-**5**B show the effect of the therapeutic enzymes on lysosomal swelling and glycogen content in GAA KO (KO) myotubes. FIG. **5**A shows confocal images of WT, untreated-(KO) and treated KO myotubes following incubation with Lumizyme (KO-Lumizyme), Endo F3 remodeled rhGAA (KO-Endo-F3), and Endo A remodeled rhGAA (KO-Endo-A). Enlarged LAMP1-positive lysosomes (red) can be detected in untreated-(KO) and in Lumizyme-treated cells but not in WT or KO myotubes treated with the remodeled enzymes. Nuclei are stained with DAPI (blue). Bar: 10 μm. FIG. **5**B shows a graph illustrates glycogen levels measured in cell lysates from WT, untreated- and treated KO myotubes. Endo F3 remodeled enzymes restored normal levels of glycogen in KO myotubes; n=5 for each condition. Statistical significance was determined by one-way ANOVA; the graph represents mean±SD. ** $p<0.01$; **** $p<0.0001$; ns—statistically insignificant.

[0022] FIG. **6**. ESI-MS profiles of Glycopeptides 59-66

[0023] FIG. **7**. ESI-MS profile of Glycoprotein 68

[0024] FIG. **8** Representative SPR binding sensorgrams of CI-MPR with M6P-containing glycopeptides. K_D values obtained from three independent experiments for compounds 62, 65 and 66 were 70.1±2.1 nM, 82.4±14.1 nM and 53.7±5.6 nM, respectively.

[0025] FIG. **9** Representative SPR binding sensorgrams of CI-MPR with remodeled RNase B. K_D value obtained from three independent experiments was 15.8±1.9 nM.

[0026] FIGS. **10**A-**10**D show the results of glycan analysis of different stages of rhGAA: native rhGAA (FIG. **10**A), rhGAA treated with Endo-A (FIG. **10**B), Endo-A remodeled rhGAA (FIG. **10**C), and Endo-F3 remodeled rhGAA (FIG. **10**D). the structures marked with dotted boxes were the residual glycans that not completely removed by buffer exchange

[0027] FIG. **11**. Transglycosylation of α1,6FucGlcNAc-CD52 with wild-type Endo F3. The reaction was complete within 30 min, and no hydrolysis of the product was observed after 5 h, indicating that Endo F3 would not cleave the newly formed glycosidic bond.

[0028] FIG. **12**. Representative SPR binding sensorgrams of different stages of rhGAA. Average K_D values for native rhGAA, Endo-A remodeled rhGAA (69) and Endo-F3 remodeled rhGAA (70) were 14.0±3.7 nM, 2.3±0.2 nM and 0.63±0.07 nM, respectively.

[0029] FIG. **13**. MALDI-TOF MS analysis of different glycoforms of rhGAA

[0030] FIG. **14**. Effect of the therapeutic enzymes on lysosomal swelling in GAA-deficient (KO) myotubes. Confocal images of WT, untreated-(KO) and treated KO myotubes exposed to Lumizyme (KO-Lumizyme), Endo-F3 remodeled rhGAA (KO-Endo-F3), and Endo-A remodeled rhGAA (KO-Endo-A). Both untreated and Lumizyme-treated KO cells contain enlarged LAMP1-positive lysosomes (red); the structures of this size are not seen in WT and KO myotubes treated with the remodeled enzymes (KO-Endo-F3 and KO-Endo-A). Nuclei are stained with DAPI (blue). Bar: 10 μm for all images.

[0031] FIG. **15** shows ESI-MS spectrum of glycoprotein 68.

[0032] FIG. **16** shows an embodiment of the one pot reaction method of the present disclosure.

[0033] FIG. **17** shows scheme 6, Stepwise and one-pot strategy to modify RNase B with the minimal tetrasaccharide oxazoline.

[0034] FIG. **18** shows scheme 7, M6P glycan remodeling of rhGAA.

DETAILED DESCRIPTION

[0035] In various embodiments, the present disclosure provides phosphorylated di-, tri-, tetra- and pentasaccharide oxazolines and their donor substrate specificity for endoglycosidases for use in glycan remodeling of peptides and proteins and methods of using such remodeled peptides and proteins as therapeutic agents. Specific phosphorylated oligosaccharide oxazolines (e.g., tetrasaccharide oxazolines) disclosed herein are used as an enzyme substrate in glycan remodeling to prepare high-affinity ligands (e.g., glycoproteins) for cation-independent mannose-6-phosphate receptor (CI-MPR) uptake by cells and the resulting glycan remodeled glycoproteins can be used as improved therapeutics. Remarkably, the glycoengineered peptides and proteins carrying the phosphorylated oligosaccharide are resistant to hydrolysis by the endoglycosidases and their mutants, which enables a simple, one-pot glycan remodeling method that combines the deglycosylation and transglycosylation reactions in one reactor without the need to separate the deglycosylated intermediate and the final protein product (for example, final enzyme product).

[0036] The methods described herein can selectively convert high-mannose type or complex type N-glycans in a multiply glycosylated protein into high-affinity M6P-oligosaccharide moieties. The method provides a general approach for a simple, one-pot glycan remodeling approach for improving any glycoprotein for enhanced cellular uptake and improved therapeutic efficacy, for example, for replacement lysosomal enzymes used in enzyme replacement therapy (ERT).

Definitions

[0037] Examples of the present disclosure have been described in terms of one or more preferred embodiments, and it should be appreciated that many equivalents, alternatives, variations, and modifications, aside from those expressly stated, are possible and within the scope of the present disclosure. Before the present disclosure is described, it is understood that embodiments provided in the present disclosure is not limited to the particular methodology, protocols, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the disclosure.

[0038] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one skilled in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the chemicals, cell lines, vectors, animals, instruments, statistical analysis and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0039] The terms"comprise(s)", "include(s)", "having", "has", "can", "contain(s)", and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments "comprising", "consisting of", and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0040] The modifier "about" used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (for example, it includes at least the degree of error associated with the measurement of the particular quantity). The modifier "about" should also be considered as disclosing the range defined by the absolute values of the two endpoints. For example, the expression "from about 2 to about 4" also discloses the range "from 2 to 4." The term "about" may refer to plus or minus 10% of the indicated number. For example, "about 10%" may indicate a range of 9% to 11%, and "about 1" may mean from 0.9-1.1. Other meanings of "about" may be apparent from the context, such as rounding off, so, for example "about 1" may also mean from 0.5 to 1.4.

[0041] Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in Organic Chemistry, Thomas Sorrell, University Science Books, Sausalito, 1999; Smith and March March's Advanced Organic Chemistry, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, Comprehensive Organic Transformations, VCH Publishers, Inc., New York, 1989; Carruthers, Some Modern Methods of Organic Synthesis, 3rd Edition, Cambridge University Press, Cambridge, 1987; the entire contents of each of which are incorporated herein by reference.

[0042] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in

addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0043] "Sugar" refers to carbohydrate-containing molecules, including, but not limited to, a monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide. A sugar molecule may be substituted, for example, by one or more phosphate groups (e.g., $(HO)_2OPO$—).

[0044] A "sugar moiety" as used herein refers to a monovalent or divalent sugar residue derived from a parent sugar molecule.

Oxazolines Compounds

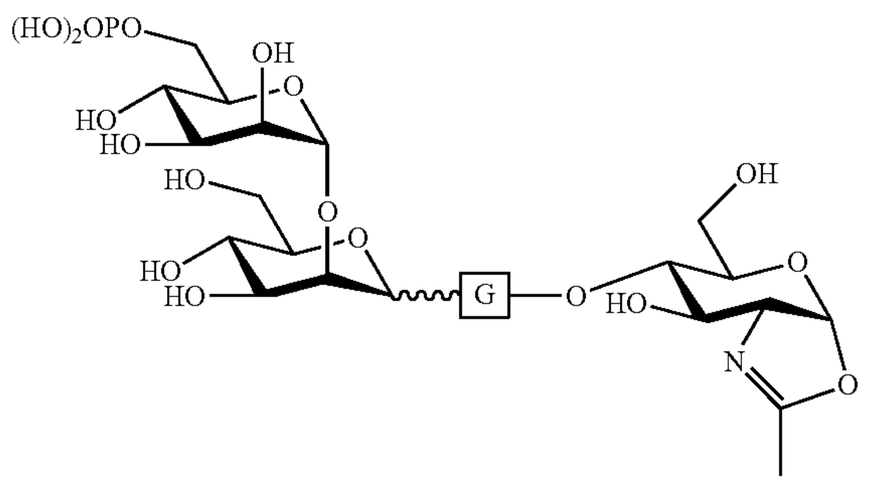
[0045] Functionalization of therapeutic glycoproteins with mannose-6-phosphate (M6P) glycan ligands represents a major strategy for enhancing the cation-independent M6P receptor (CI-MPR)-mediated cellular uptake, thus improving the overall therapeutic efficacy of the glycoproteins including when being administered to a subject. The present disclosure provides synthetic phosphorylated oxazolines, for example, M6P-containing N-glycan oxazolines, that can be used as donor substrates for transglycosylation to a therapeutic moiety (e.g., glycoprotein) to a make an M6P-containing glycoprotein that can use to target cellular uptake (via CI-MPR) in a cell. The synthetic phosphorylated oxazolines described herein can be used in a one-step method to remodel the glycans on the glycoprotein, and thus provides a "one step" method of conjugating glycoprotein therapeutic molecules.

[0046] In the present disclosure, the inventors demonstrate a minimal high-affinity M6P-containing N-glycan ligand for efficient and site-selective conjugation to therapeutic glycoproteins. The chemical synthesis of truncated M6P-glycan oxazolines and their use for enzymatic glycan remodeling of recombinant proteins (for example, human acid α-glucosidase (rhGAA), an enzyme used for treatment of Pompe disease). Structure-activity relationship studies identified an M6P tetrasaccharide oxazoline as the minimal substrate for enzymatic transglycosylation yielding high-affinity M6P glycan ligands for the CI-MPR. Taking advantage of the substrate specificity of endoglycosidases Endo-A and Endo-F3, we found that Endo-A and Endo-F3 could efficiently deglycosylate the respective high-mannose and complex type N-glycans in the glycoprotein and site-selectively transfer the synthetic M6P N-glycan to the deglycosylated glycoprotein without product hydrolysis. This discovery enabled a highly efficient one-pot deglycosylation/transglycosylation strategy for site-selective M6P-glycan remodeling of glycoproteins to obtain a more homogeneous product. The Endo-A and Endo-F3 remodeled glycoproteins maintained full enzyme activity and demonstrated 6- and 20-fold enhanced binding affinities for CI-MPR receptor, respectively.

[0047] The synthesis of several M6P-containing N-glycan oxazolines (compounds 1-4, FIG. **1**) was previously reported and the synthesized compounds were used as donor substrates for endoglycosidase-catalyzed chemoenzymatic synthesis of M6P-containing glycoproteins, using ribonuclease B as a model glycoprotein.[30] Binding studies have revealed that a single M6P moiety located at the low α-1,3-branch of the N-glycan context, derived from glycan oxazoline 3, is sufficient for a high-affinity binding to CI-MPR, while the presence of an M6P moiety at the α-1,6-branch, as shown in glycan oxazolines 1 and 2, appears dispensable.[30] Despite these findings, the substrate specificity of the M6P-glycan oxazolines in enzymatic transglycosylation and the detailed structure-activity relationship of the M6P glycan ligands for receptor binding remained to be elucidated. In addition, it was not clear what constitutes the minimal M6P-glycan structures for high-affinity CI-MPR binding feasible for a facile enzymatic glycan remodeling of therapeutic lysosomal enzymes. To address these problems, the present disclosure provides the chemical synthesis and evaluation of a series of truncated M6P-containing N-glycan oxazolines, including for example compounds 5-10 (FIG. **1**), which may be used as donor substrates for enzymatic transglycosylation and glycan remodeling of glycoproteins, such as therapeutic lysosomal enzymes (e.g., commercial rhGAA, Lumizyme).[31]

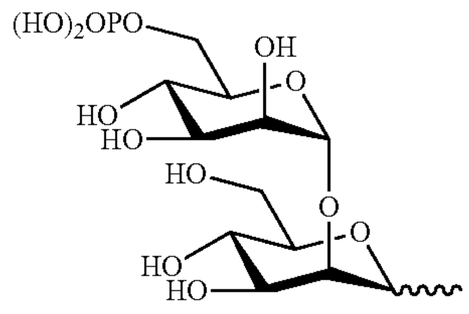
[0048] In one aspect, the present disclosure provides a compound of Formula (I), or a salt thereof,

(I)



[0049] wherein G is a sugar moiety or a linker.

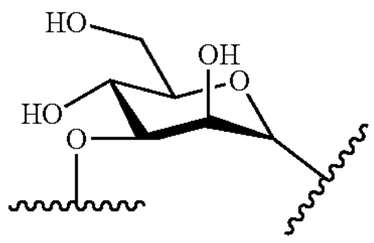
[0050] In some embodiments, G is a linker, such as a single bond, a sugar moiety, PEG linker, or a triazole linker. For example, the linker may be a triazole linker formed by azide-alkyne cycloaddition ("click chemistry"). In some embodiments, the linker is not a sugar moiety (or a non-sugar linker). The sugar moiety may, for example, be a linear sugar moiety or a branched sugar moiety. In some preferred embodiments, the sugar moiety is a linear moiety. In general, G may be any structure that does not interfere with or block the activity of an endoglycosidase as disclosed herein. For example, the mannose-6-phosphate-α-1,2-mannose (Man6Pα1,2Man) moiety may provide a binding motif for the endoglycosidase, G may have a structure that does not block or hinder the binding of a compound of Formula (I) to the endoglycosidase, and the endoglycosidase may effectively use the compound of Formula (I) as a substrate.



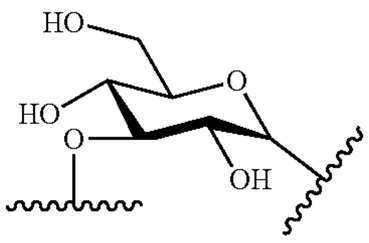
(Man6Pa1,2Man moiety)

[0051] In some embodiments, G is a sugar moiety, such as a monosaccharide moiety, a disaccharide moiety, a trisaccharide moiety, or a tetrasaccharide moiety. In some embodiments, G is a linear sugar moiety. Suitable linear sugar moieties include, for example, a monosaccharide, linear disaccharide, linear trisaccharide or linear tetrasaccharide moiety. In some embodiments, G is not a branched sugar moiety (e.g., compounds of Formula I do not include compound 1 or 3 found in FIG. **1**). In some embodiments, G is a monosaccharide moiety, such as a mannose moiety or a glucose moiety. The monosaccharide moiety may be attached to the remainder of the parent molecule through a 1,2-divalent linkage, a 1,3-divalent linkage, a 1,4-divalent linkage, a 1,6-divalent linkage, a 2,3-divalent linkage, a 2,4-divalent linkage, a 2,6-divalent linkage, a 3,4-divalent linkage, a 3,6-divalent linkage, or a 4,6-divalent linkage. In some embodiments, the monosaccharide moiety is a 1,3-mannose moiety or a 1,3-glucose moiety (shown below). The bond at C1 of monosaccharide moiety may be a 1α or 1β configuration.

[0052] Linkers include other natural glycosidic bonds, for example, but not limited to, PEG linker, triazole click linker, etc. An alternative approach to that described herein includes the transfer of an azide-tagged sugar, then click a high-affinity M6P-glycan ligand.

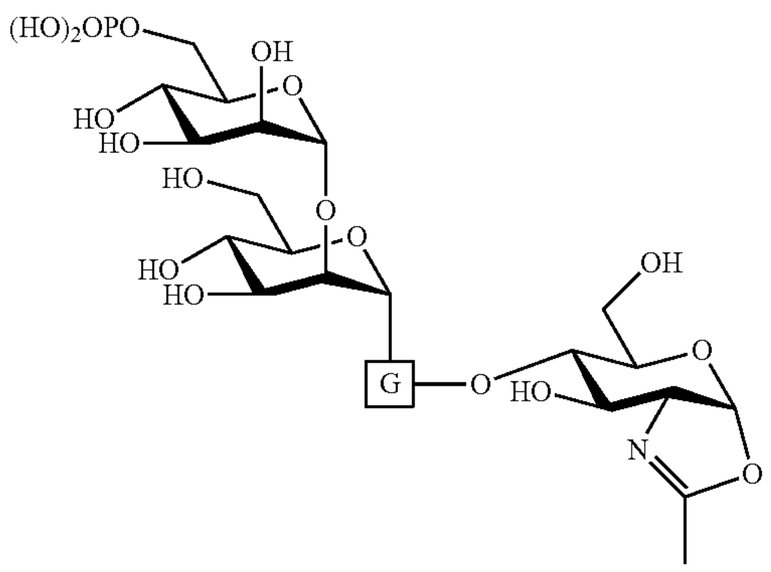


1,3-mannose moiety



1,3-glucose moiety

[0053] In some embodiments, the compound of Formula (I) has a structure of Formula (I-a), or a salt thereof



(I-a)

[0054] wherein G is as defined in Formula (I).

[0055] Suitable compound of Formula (I) may be selected from the group consisting of

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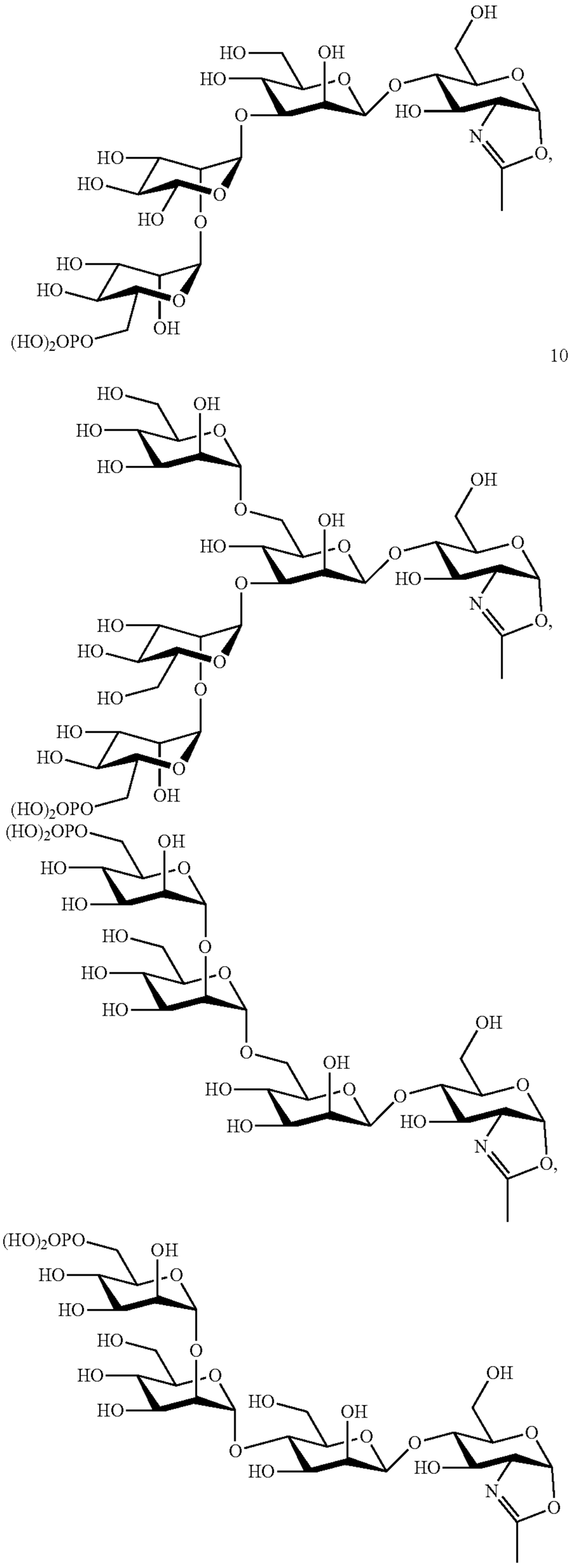

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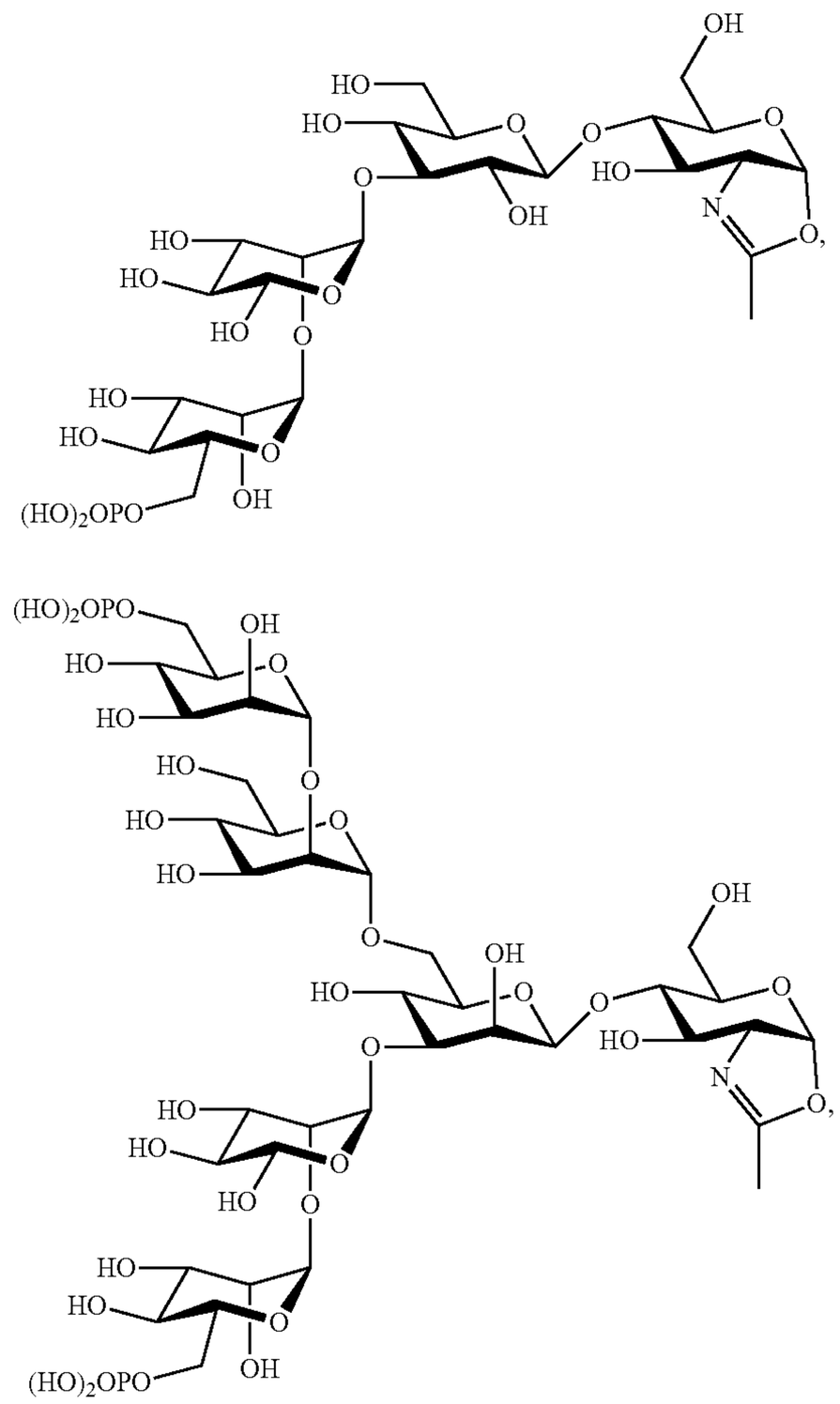

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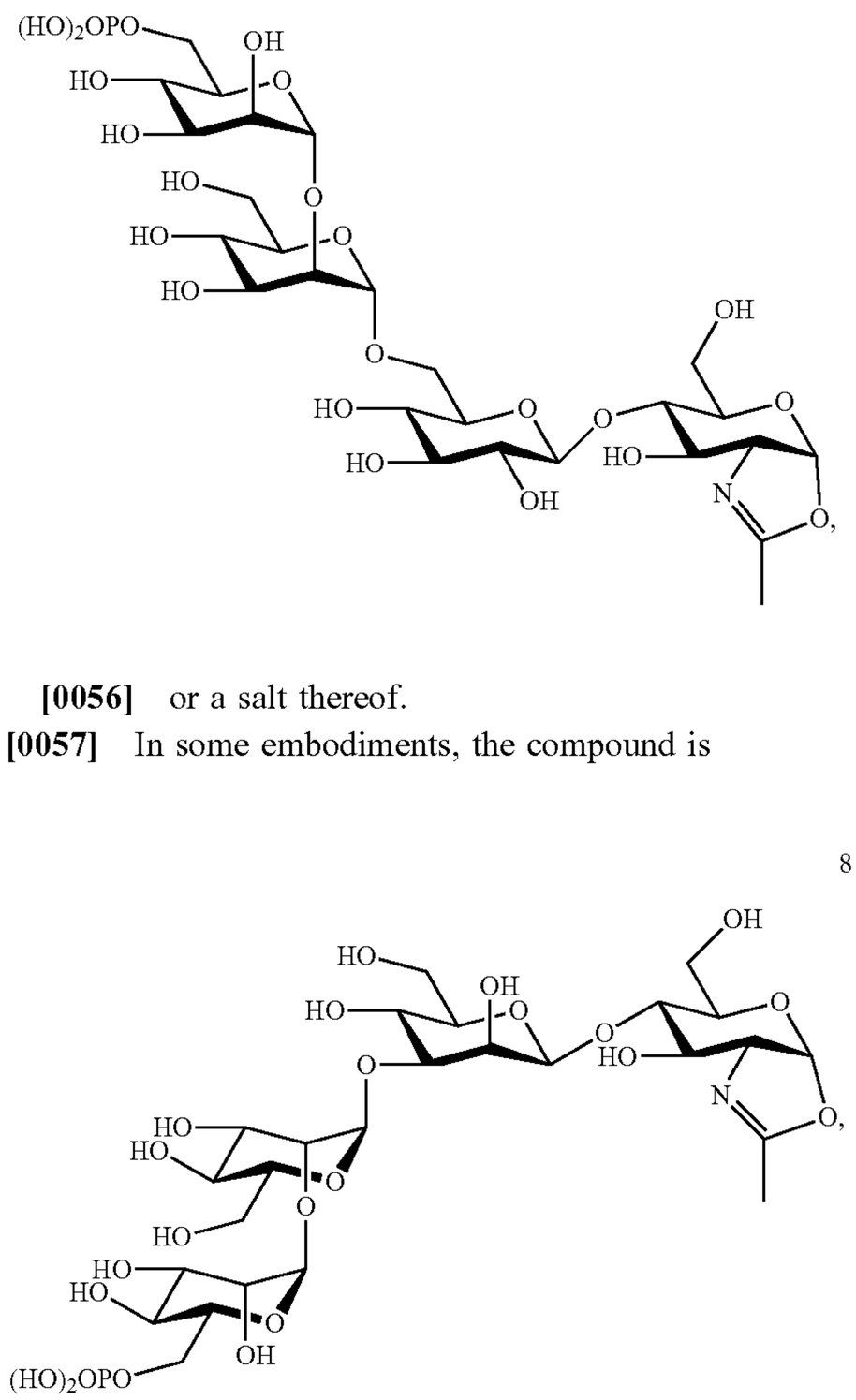


[0056] or a salt thereof.

[0057] In some embodiments, the compound is

[0058] or a salt thereof.

[0059] The salt may be, for example, a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salt" refers to salts derived from a variety of organic and inorganic counter ions known in the art. Pharmaceutically acceptable acid addition salts can be formed with inorganic acids and organic acids. Inorganic acids from which salts can be derived include, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, and phosphoric acid. Organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, and salicylic acid. Pharmaceutically acceptable base addition salts can be formed with inorganic and organic bases. Inorganic bases from which salts can be derived include, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, and aluminum. Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic ami-

nes, and basic ion exchange resins. Specific examples include isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. In some embodiments, the pharmaceutically acceptable base addition salt is chosen from ammonium, potassium, sodium, calcium, and magnesium salts.

[0060] The present disclosure further provides remodeled glycoproteins. The remodeled glycoproteins comprise one or more of the compounds (e.g., glycan oxazolines) described herein. The glycoprotein may be any protein or peptide that comprises a modifiable sugar (e.g., mannose or N-glycans) that is able to bind endoglycosidase that is capable of deglycosylation and transglycosylation in the same reaction mixture, and without a purification of an intermediate. In some embodiments, the remodeled glycoprotein is remodeled to include a mannose-6-phosphate group, which is capable of specific binding to its receptor on cell surfaces.

Method of Remodeling Glycoprotein

[0061] The present disclosure also relates to a method for selectively converting high-mannose type or complex type N-glycans in a multiply glycosylated protein into high-affinity M6P-oligosaccharide moieties. The method provides a general approach for a simple, one-pot glycan remodeling approach for glycoproteins, such as therapeutic lysosomal enzymes, thereby enhancing their cellular uptake and overall therapeutic efficacy. These methods provide a one pot process to increase the yield, reduce purification steps and provide a purified product that, in some embodiments, has increased therapeutic efficacy. Further, this procedure allows for the ability to target the therapeutic to the lysosymal compartments in a cell.

[0062] In particular, the method may employ high-affinity tetrasaccharide oxazoline and wild type endoglycosidases, such as Endo-A from *Arthrobacter protophormia* or Endo F3 from *Elizabethkingia meningoseptica* to perform transglycosylation on multiply glycosylated proteins without hydrolysis of the resulting products. In specific embodiments, the remarkable difference in hydrolytic activity of the wild-type Endo-A/Endo-F3 toward the parent enzyme and the resulting transglycosylation product enables a simple, one-pot process that combines the protein deglycosylation and transglycosylation without the need to separate the deglycosylation intermediate and the enzyme. This method can selectively convert high-mannose type or complex type N-glycans in a multiply glycosylated protein into high-affinity M6P-oligosaccharide moieties, thus can be used to enhance cellular uptake and overall therapeutic efficacy of any glycoprotein, for example, lysosomal enzymes used in enzyme replacement therapy (ERT).

[0063] In one aspect, the present disclosure provides a method for remodeling a glycoprotein, comprising: a) contacting the glycoprotein with an endoglycosidase thereby producing a deglycosylated intermediate comprising a N-acetylglucosamine (GlcNAc) or core-fucosylated N-acetylglucosamine (Fuca1,6GlcNAc) acceptor from the glycoprotein by a deglycosylation activity of the endoglycosidase to produce a deglycosylated intermediate; and (b) contacting a glycan oxazoline comprising a mannose-6-phosphate (M6P) moiety with the deglycosylated intermediate in the presence of the endoglycosidase, thereby attaching the glycan oxazoline to the N-acetylglucosamine (GlcNAc) or core-fucosylated N-acetylglucosamine (Fuca1, 6GlcNAc) acceptor by a transglycosylation activity of the endoglycosidase, thereby producing a remodeled glycoprotein, wherein (a) and (b) are carried out in a one-pot reaction (e.g., in a single reaction mixture without any purification steps to remove the endoglycosidase). In some embodiments, the endoglycosidase is selected from the group consisting of wild type Endo A, wild type Endo F3, wild type Endo-CC, and a combination of. Further, the resultant remodeled glycoprotein, specifically, enzymatic glycoproteins and especially lysosomal enzymes are capable of being targeted to the lysozyme through the specific and increased binding to the CI-MPR receptor.

[0064] The term "one-pot reaction" refers to a reaction which is performed without isolating or separating any intermediate product. In other words, the intermediate product is produced and then used in situ, as the term is understood in the art, for the next step of the reaction. Routine experimental procedures that do not remove the intermediate product from the reaction mixture (e.g., evaporation of solvent, dialysis) may be included in a one-pot reaction. The order in which reagents for a one-pot reaction (e.g., starting materials, enzymes, substrates) are added are not limited. For example, the reagents may be added sequentially as the reaction progresses or the reagents may be added all together at the beginning of the reaction.

[0065] The one pot method described herein can be used for any glycoprotein (e.g., protein that contains one or more modifiable sugar moieties). The glycoproteins only requirement is to have one or more modifiable sugar moieties, as demonstrated in the examples and described herein. For example, the one or more sugar moieties may be any N-glycans, including high-mannose type, complex type, hybrid type, or their truncated forms.

[0066] The glycoproteins may be proteins or peptides that are used as therapeutics and targeted to the lysosyme. In some embodiments, the glycoproteins are enzymatic proteins. In other embodiments, the glycoproteins may be any protein that is to be targeted to lysozymes (e.g., enzymes).

[0067] In some embodiments, the glycoprotein is a lysosomal enzyme. For example, the lysosomal enzyme may be a therapeutic enzyme for enzymatic replacement therapy (ERT). In some embodiments, the present method remodels of the lysosomal enzyme, resulting in site-selective introduction of a high-affinity ligand (e.g., an M6P ligand), which improves the therapeutic efficacy of the therapeutic enzyme.

[0068] Suitable lysosomal enzymes include, but are not limited to, α-galactosidase A, acid ceramidase, acid α-L-fucosidase, acid β-glucosidase, acid β-galactosidase, iduronate-2-sulfatase, α-L-iduronidase, galactocerebrosidase, acid α-mannosidase, acid β-mannosidase, arylsulfatase B, arylsulfatase A, N-acetylgalactosamine-6-sulfate sulfatase (N-acetylgalactosamine-6-sulfatase, or galactose-6-sulfatase), acid β-galactosidase, acid sphingomyelinase, acid α-glucosidase (α-glucosidase), β-hexosaminidase B, heparan N-sulfatase, α-N-acetylglucosaminidase, acetyl-CoA: α-glucosaminide N-acetyltransferase, N-acetylglucosaminide-6-sulfate sulfatase, α-N-acetylgalactosaminidase, sialidase, β-glucuronidase, β-hexosaminidase A, and a combination thereof. In some embodiments, the lysosomal enzyme comprises at least one asparagine (N)-linked glycan. In some embodiments, the lysosomal enzyme is an acid α-glucosidase (α-glucosidase).

[0069] For example, Lumizyme® (alglucosidase alfa, Genzyme Corporation) is a therapeutic lysosomal enzyme that carries 7 different N-glycans and currently is used for the treatment of Pompe disease. The structure-activity relationship study revealed a Man6P-α1,2-Man disaccharide moiety in the synthetic N-glycans as a structural motif for high-affinity binding to the CI-MPR and identified a tetrasaccharide oxazoline as a donor substrate for enzymatic transglycosylation to provide high-affinity M6P glycan ligands for the CI-MPR. In specific embodiments, the present disclosure provides a method for one-pot fand site-selective glycan remodeling of the multiply glycosylated rhGAA to produce a more homogeneous glycoengineered enzyme that showed up to 20-fold enhanced binding affinities for the CI-MPR over the commercial Lumizyme. The present disclosure also demonstrates significantly enhanced cellular uptake of M6P-glycan remodeled rhGAA in a cell model system for Pompe disease, leading to much more efficient degradation of glycogen in lysosomes than the commercial Lumizyme under the same conditions.

[0070] The endoglycosidase as disclosed herein may have a deglycosylation activity, transglycosylation activity, or both. In some embodiments, the endoglycosidase is wild type Endo A. The wild type Endo A may comprise a sequence of SEQ ID NO:1. In some embodiments, the wild type Endo A removes high-mannose and hybrid type glycans from a glycoprotein (e.g., a lysosomal enzyme) without affecting complex-type glycans.

[0071] In some embodiments, the endoglycosidase is wild type Endo F3. The wild type Endo F3 may comprise a sequence of SEQ ID NO:2. In some embodiments, the wild type Endo F3 removes core-fucosylated complex-type glycans from the glycoprotein (e.g., lysosomal enzyme) without affecting high-mannose or hybrid type glycans.

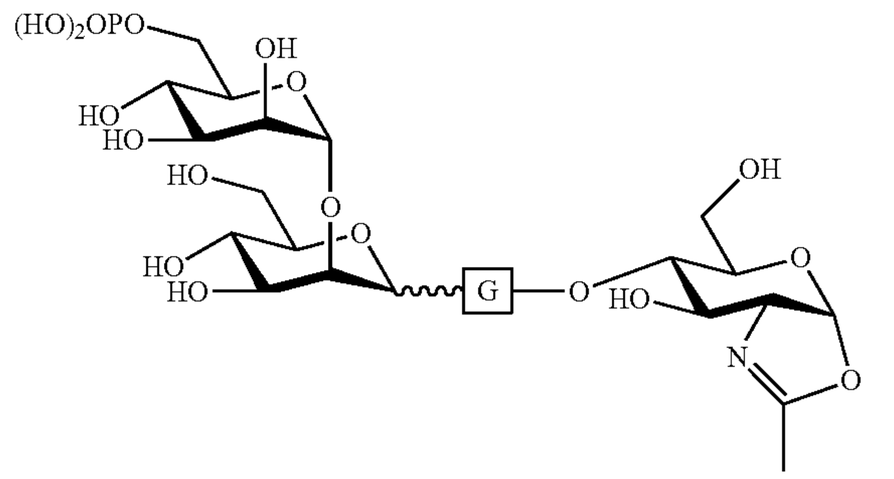
[0072] In some embodiments, the endoglycosidase is wild type Endo-CC. The wild type Endo-CC may comprise a sequence of SEQ ID NO:3. In some embodiments, the wild type Endo-CC removes high-mannose type and biantennary complex type glycans from the glycoprotein (e.g., lysosomal enzyme) without affecting core-fucosylated complex-type glycans or higher branched complex type glycans.

[0073] In some embodiments, the endoglycosidase is a combination of the wild type Endo A and the wild type Endo F3. The ratio of the activity of the wild type Endo A (Unit) to that of the wild type Endo F3 in the combination may be about 0.1:99.9 to about 99.9:0.1, such as about 1:99, about 10:90, about 20:80, about 30:70, about 40:60, about 50:50, about 60:40, about 70:30, about 80:20, about 90:10, or about 99:1.

[0074] The glycan oxazoline may be any suitable compound comprising a mannose-6-phosphate (M6P) moiety that can be used as a glycan donner substrate in the transglycosylation reaction mediated by the endoglycosidase. In some embodiments, the glycan oxazoline comprises at least one Man6Pa1,2Man moiety, as disclosed herein.

[0075] In some embodiments, the glycan oxazoline has a structure of formula (I), or a salt thereof,

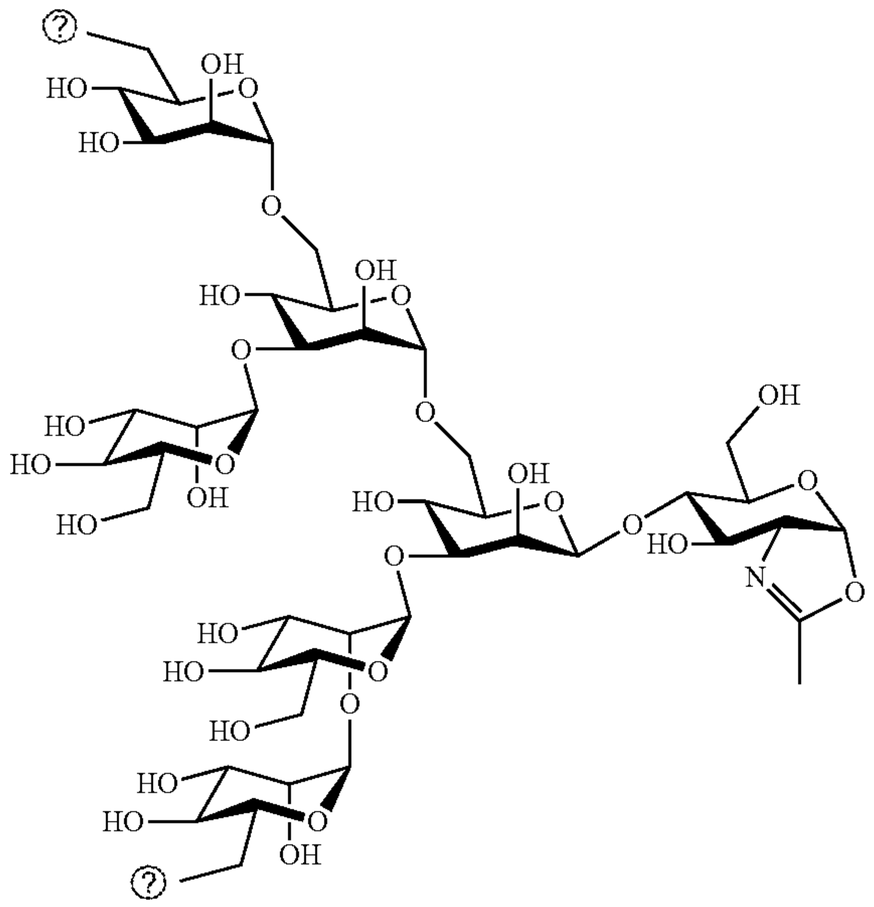
(I)



[0076] wherein G is sugar moiety or a linker, as disclosed herein.

[0077] In some embodiments, G is a sugar moiety, such as a monosaccharide moiety, a disaccharide moiety, a trisaccharide moiety, or a tetrasaccharide moiety. In some embodiments, G is a monosaccharide moiety, such as a mannose moiety or a glucose moiety as disclosed herein. In some embodiments, G is a linker that links the two sugar moieties via a bond.

[0078] Suitable glycan oxazoline compounds include, for example, one selected from the group consisting of:



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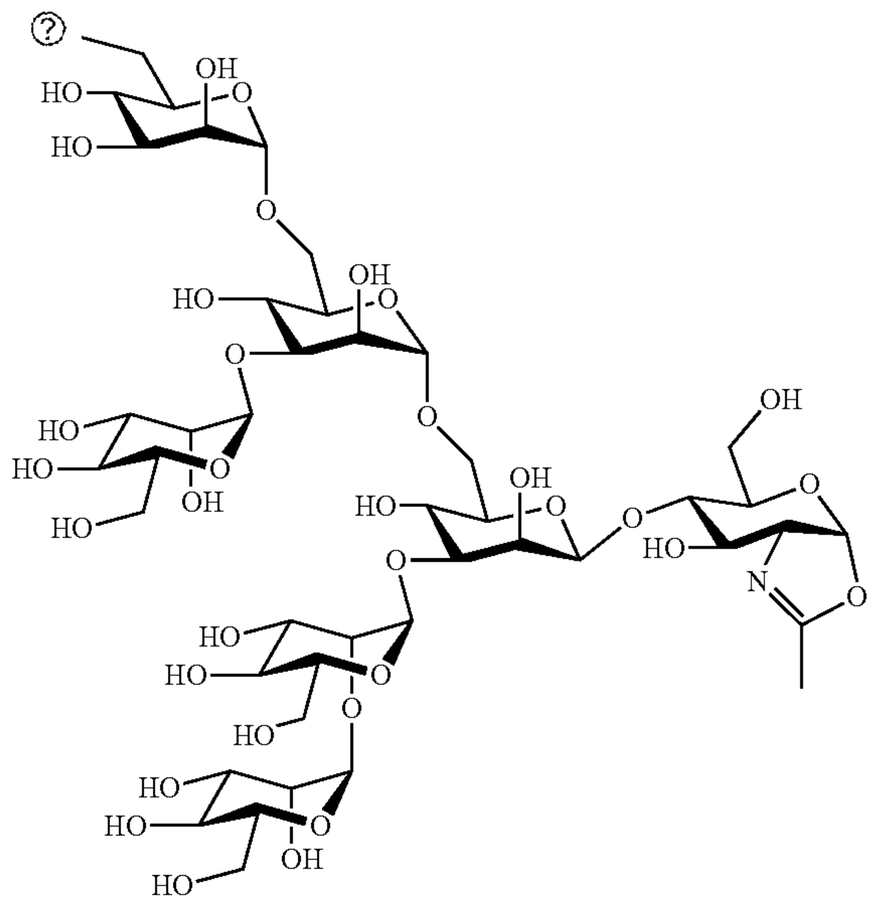

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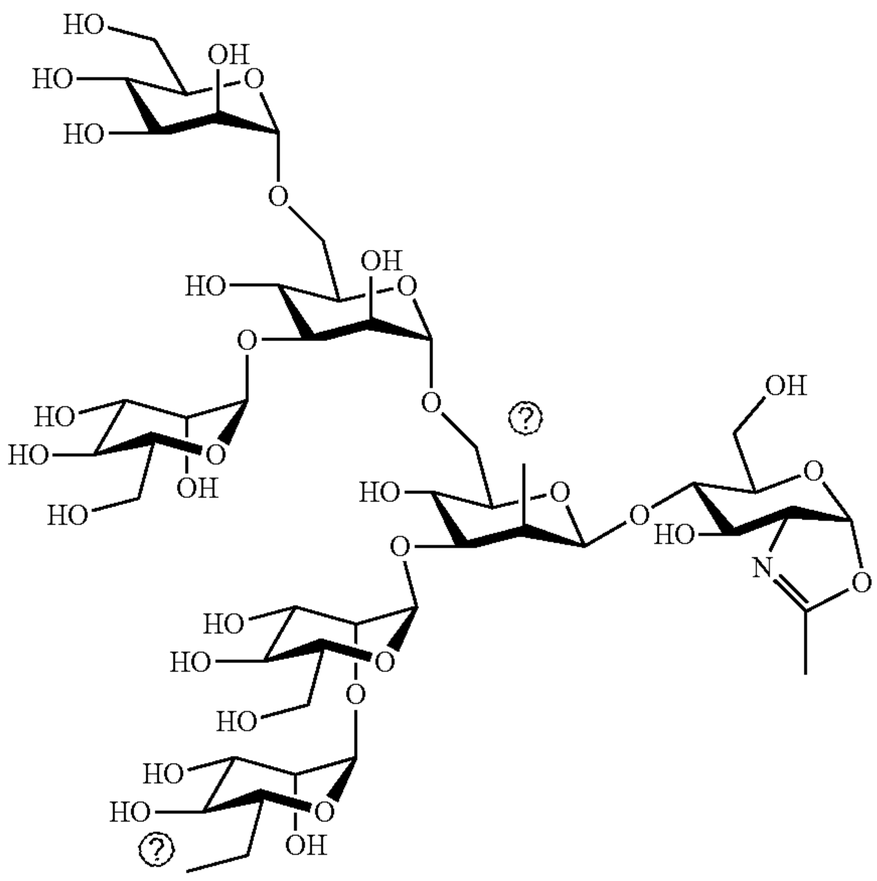

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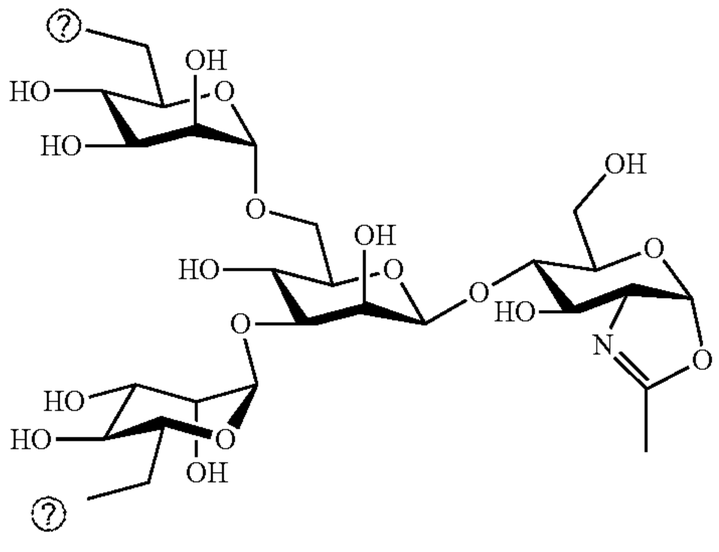

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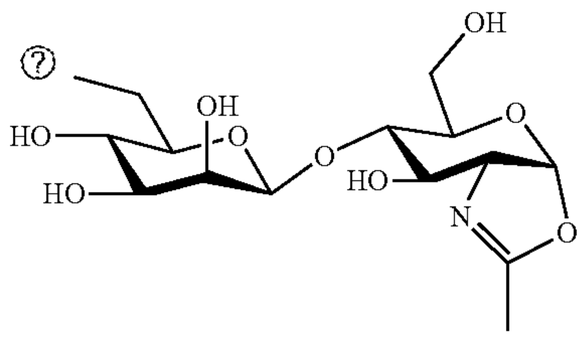

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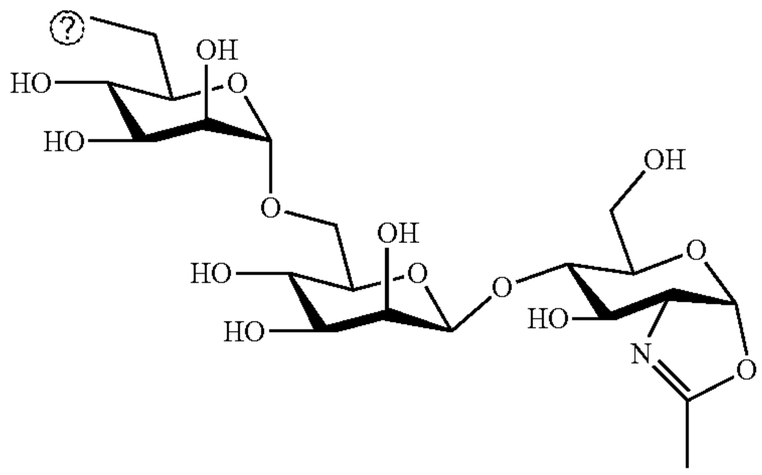

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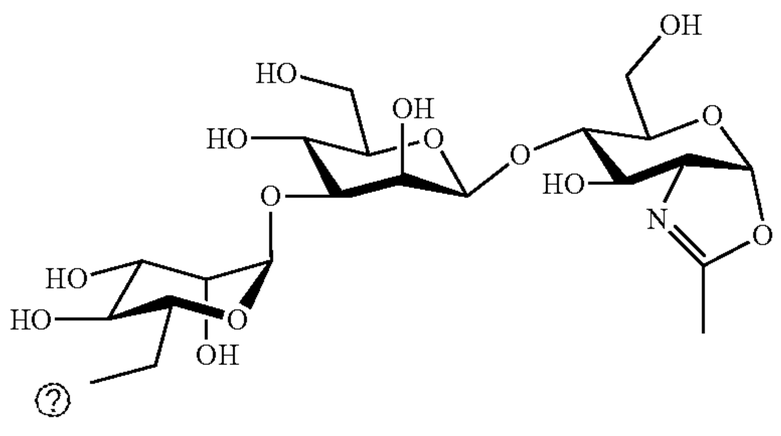

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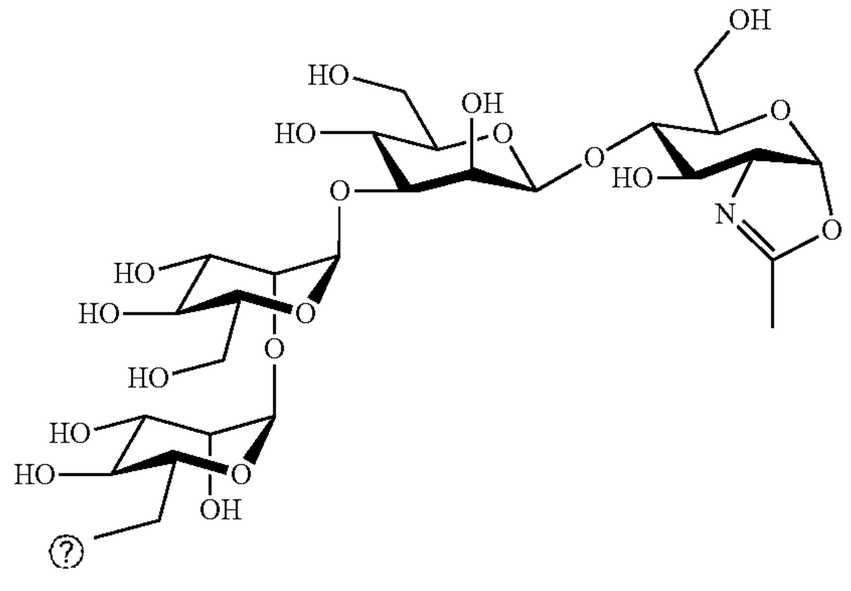

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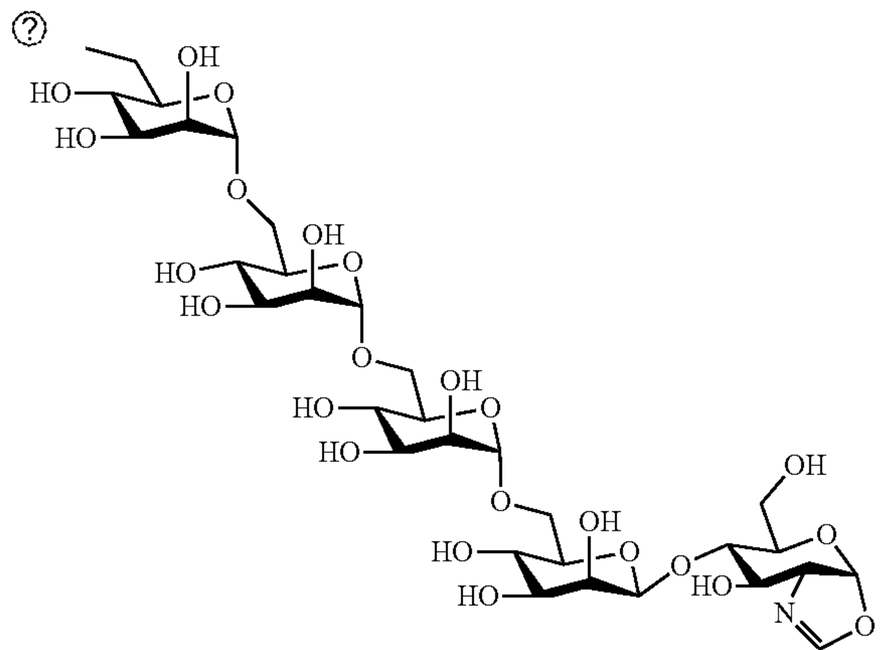

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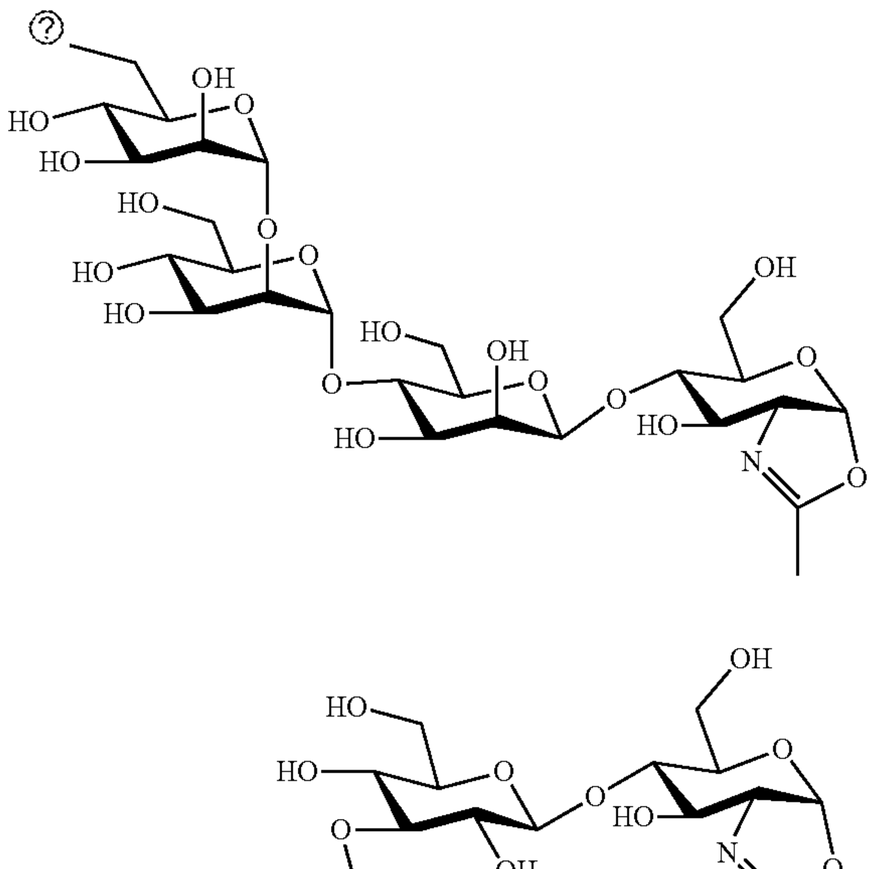

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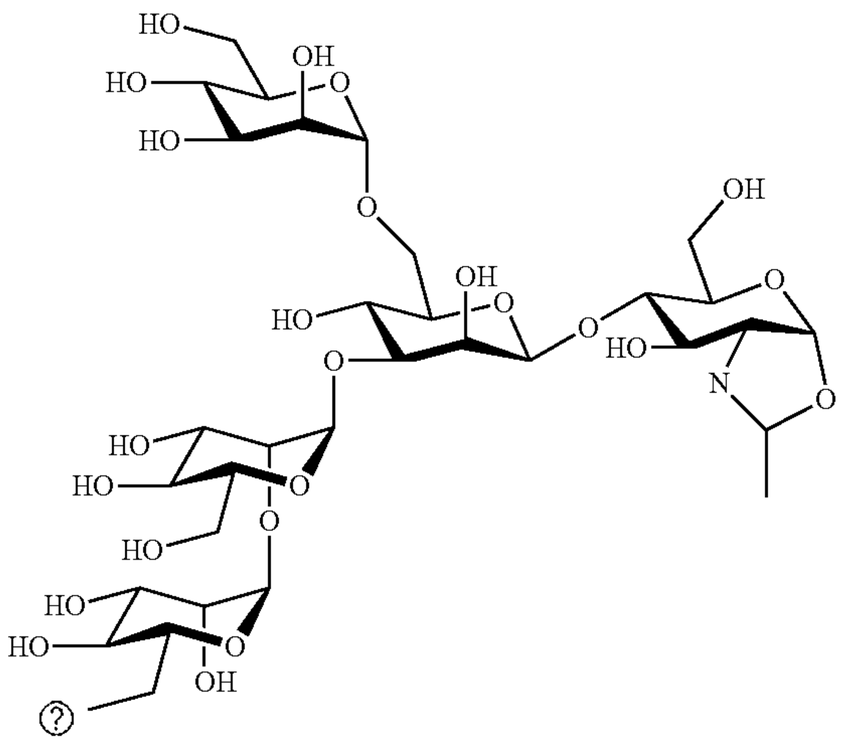

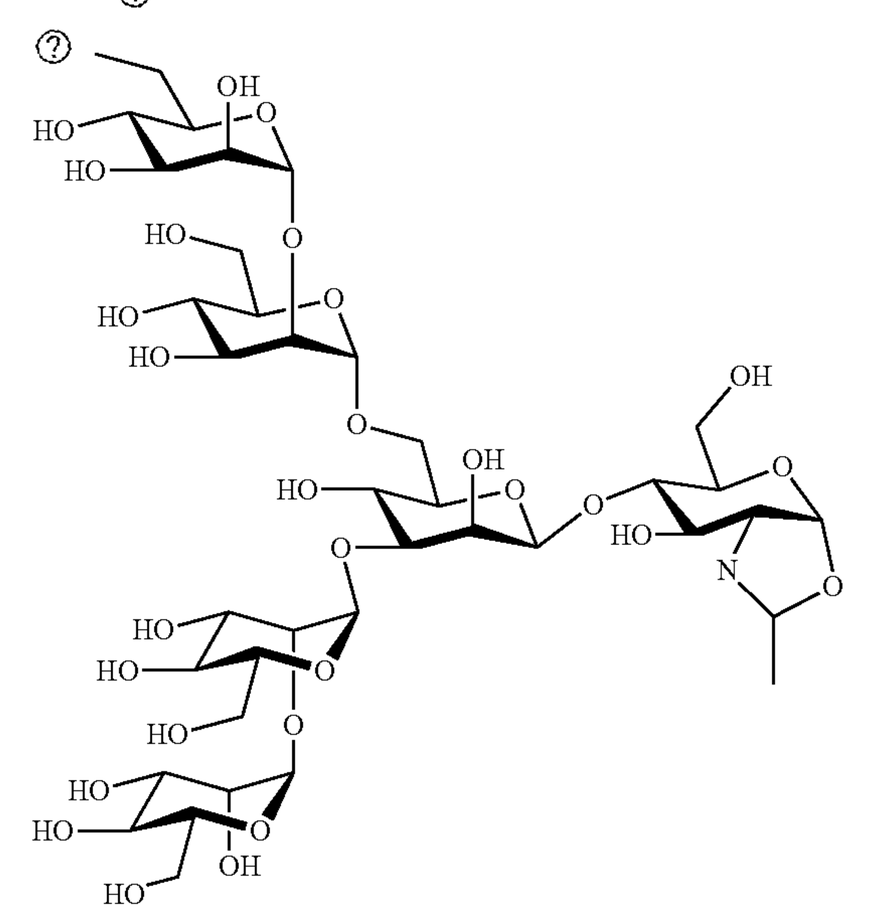

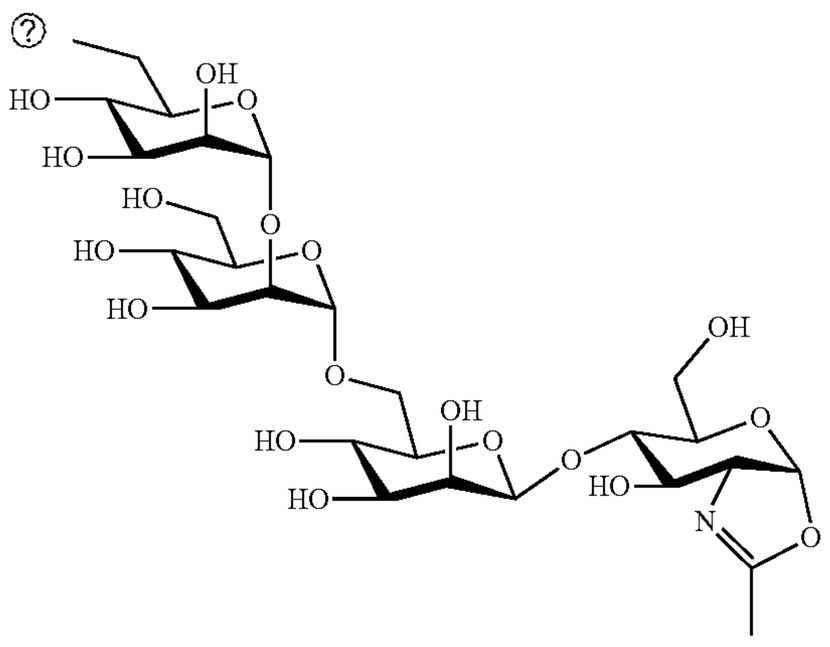

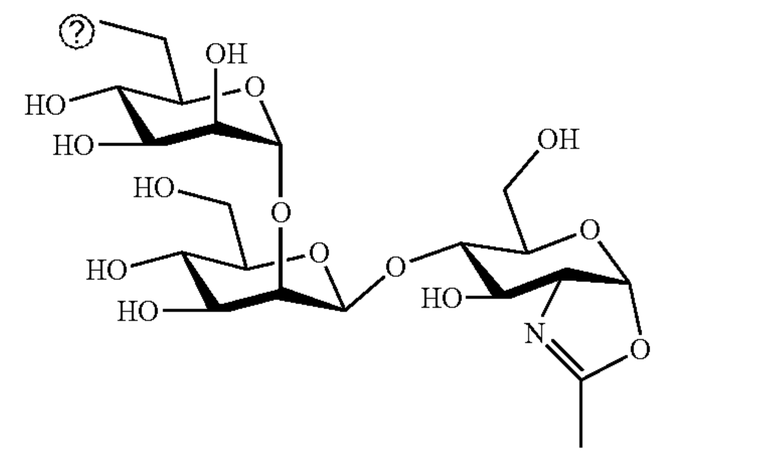

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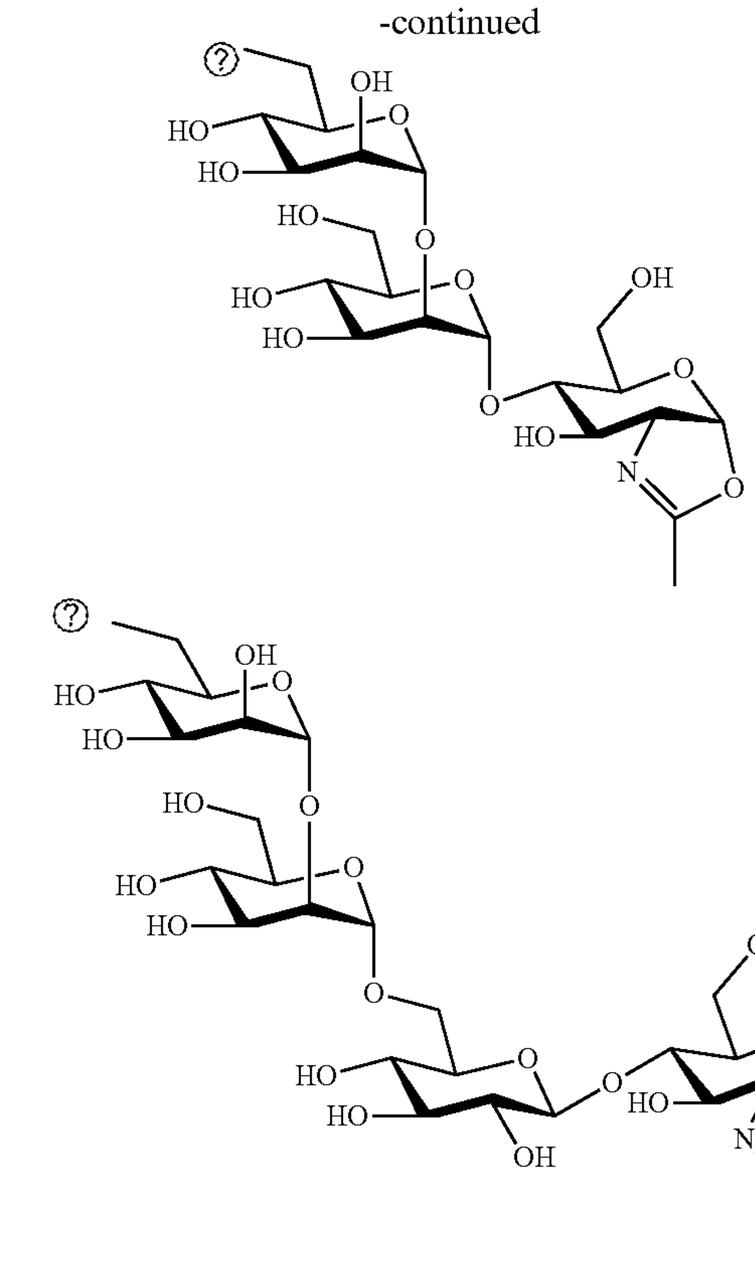

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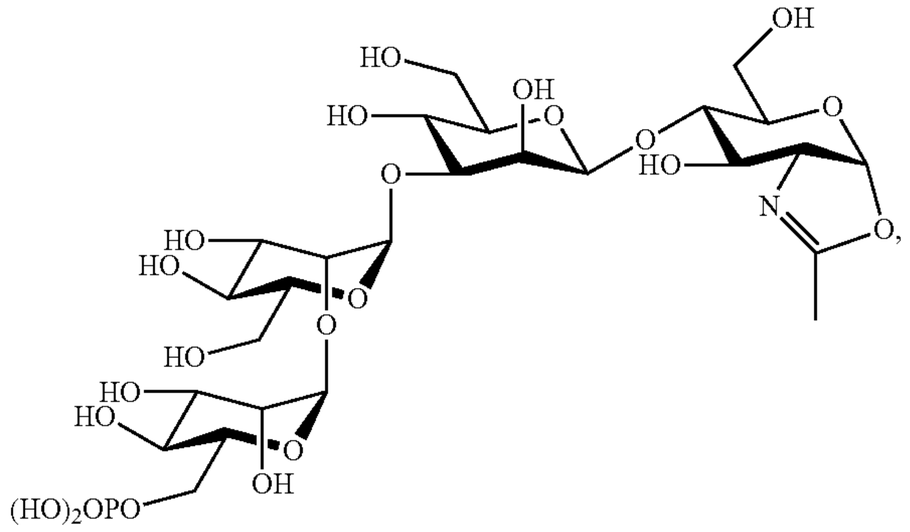
[0079] or a salt thereof.

[0080] In some embodiments, the glycan oxazoline is



[0081] or a salt thereof.

[0082] The present disclosure also provides remodeled glycoproteins comprising one or more of the glycan oxazoline linked to a mannose-6 phosphate (M6P) moiety as described herein. In one embodiment, provided is a method of enhancing binding affinity of a glycoprotein to a cation-independent M6P receptor (CI-MPR), comprising remodeling the glycoprotein according to the method described herein; and contacting the remodeled glycoprotein with a cell comprising CI-MPR receptor, thereby enhancing binding affinity of the glycoprotein to the CI-MPR.

[0083] The remodeled glycoprotein may have a binding affinity to CI-MPR that is at least 25% higher than that of the original glycoprotein (i.e. without glycan remodeling). For example, the binding affinity of the remodeled glycoprotein may be at least 50%, at least 100%, at least 200%, at least 500%, at least 1000%, at least 5000%, or at least 10000% higher than that of the original glycoprotein.

[0084] In another embodiment, provided is a method of enhancing or increasing uptake of a glycoprotein in a cell, comprising remodeling the glycoprotein according to the method described herein, and contacting the cell with the remodeled glycoprotein, thereby enhancing uptake of the glycoprotein in the cell.

[0085] The uptake of the remodeled glycoprotein by the cell may be at least 25% higher than that of the original glycoprotein (i.e. without glycan remodeling). For example, the uptake of the remodeled glycoprotein by the cell may be at least 50%, at least 100%, at least 200%, at least 500%, at least 1000%, at least 5000%, or at least 10000% higher than that of the original glycoprotein.

[0086] Suitable glycoproteins for these methods may include, but are not limited to lysosomal enzymes, such as acid «-glucosidase (α-glucosidase). For example, a lysosomal enzyme remodeled by the present method may have an increased binding affinity to CI-MPR and/or an uptake by the cell that is at least 100% higher (such as about 500%, about 1000%, about 2000%, or about 5000% higher) than the corresponding value of the original lysosomal enzyme.

[0087] The cell may be in vitro or in vivo. The cell may be derived from a tissue or a cell line, such as cultured cells. In some embodiments, the cell is from a mammalian cell line. For example, the cell may be a heart cell, a brain cell, a muscle cell, a liver cell, or an endothelium cell. In some embodiments, the cell is a diseased cell, such as a cell model for Pompe disease, heart disease, or cancer. In some embodiments, the lysosomal enzyme is acid α-glucosidase (α-glucosidase), and the cell is a muscle cell.

Remodeled Glycoproteins

[0088] In another aspect, provided is glycan-remodeled glycoprotein produced by the remodeling method as described herein. The glycan-remodeled glycoproteins include, but are not limited to glycan-remodeled enzymes, including, for example, lysosomal enzymes. In some embodiments, the glycan-remodeled lysosomal enzyme is a glycan-remodeled acid α-glucosidase (α-glucosidase). The glycan-remodeled glycoproteins may be targeted to the lysosomal compartments of cells.

[0089] The glycan-remodeled glycoproteins (e.g., the glycan-remodeled lysosomal enzyme) as described herein may be used as a therapeutic enzyme. In one aspect, the present disclosure also provides a pharmaceutical composition comprising a therapeutically effective amounts of a glycan-remodeled glycoproteins (e.g., a glycan-remodeled lysosomal enzyme) as described herein and a pharmaceutically acceptable carrier.

[0090] A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as beneficial or desirable biological and/or clinical results. A therapeutically effective amount of the composition may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the composition

to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the present pharmaceutical composition are outweighed by the therapeutically beneficial effects.

[0091] For example, a therapeutically effective amount of a glycan-remodeled glycoproteins (e.g., a glycan-remodeled lysosomal enzyme) of the present disclosure, may be about 1 mg/kg to about 1000 mg/kg, such as about 5 mg/kg to about 1000 mg/kg, about 10 mg/kg to about 1000 mg/kg, about 10 mg/kg to about 800 mg/kg, about 10 mg/kg to about 400 mg/kg, about 10 mg/kg to about 200 mg/kg, about 10 mg/kg to about 100 mg/kg, about 20 mg/kg to about 800 mg/kg, about 20 mg/kg to about 400 mg/kg, about 20 mg/kg to about 200 mg/kg, about 30 mg/kg to about 600 mg/kg, or about 30 mg/kg to about 300 mg/kg. The therapeutically effective amount may be, for example, about 1 mg/kg, about 10 mg/kg, about 20 mg/kg, about 50 mg/kg, about 100 mg/kg, about 200 mg/kg, about 500 mg/kg, or about 800 mg/kg. In some embodiments, the therapeutically effective amount is about 20 mg/kg.

[0092] The term "pharmaceutically acceptable carrier," as used herein, means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Suitable pharmaceutically acceptable carriers include, but are not limited to, diluents, preservatives, solubilizers, emulsifiers, liposomes, nanoparticles and adjuvants. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as, but not limited to, lactose, glucose and sucrose; starches such as, but not limited to, corn starch and potato starch; cellulose and its derivatives such as, but not limited to, sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as, but not limited to, cocoa butter and suppository waxes; oils such as, but not limited to, peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols; such as propylene glycol; esters such as, but not limited to, ethyl oleate and ethyl laurate; agar; buffering agents such as, but not limited to, magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as, but not limited to, sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

[0093] Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.01 to 0.1 M and preferably 0.05M phosphate buffer or 0.9% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of nonaqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include isotonic solutions, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media.

[0094] Compositions of the present disclosure may include liquids, lyophilized, or otherwise dried formulations and may include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the polypeptide, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, etc., or onto liposomes, microemulsions, micelles, milamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils).

[0095] The compositions can be sterilized by conventional, well-known sterilization techniques. The compositions may contain pharmaceutically acceptable additional substances as required to approximate physiological conditions such as a pH adjusting and buffering agent, toxicity adjusting agents, such as, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, and the like.

[0096] The disclosed glycan-remodeled glycoproteins (e.g., glycan-remodeled lysosomal enzyme) may be formulated for administration by, for example, solid dosing, eyedrop, in a topical oil-based formulation, injection, inhalation (either through the mouth or the nose), implants, oral, buccal, parenteral, or rectal administration. Techniques and formulations may generally be found in "Remington's Pharmaceutical Sciences", (Meade Publishing Co., Easton, Pa.). Therapeutic compositions typically are sterile and stable under the conditions of manufacture and storage.

[0097] The route by which the present composition is administered and the form of the composition may dictate the type of carrier to be used. The composition may be administered, for example, by oral, rectal, sublingual, parenteral, or topical administration. Parenteral administration may include, for example, intramuscular, intraperitoneal, intravenous, and transdermal administration. The composition may be in a variety of forms, suitable, for example, for systemic administration (e.g., oral, rectal, sublingual, buccal, implants, or parenteral) or topical administration (e.g., dermal, pulmonary, nasal, aural, ocular, liposome delivery systems, or iontophoresis).

[0098] Carriers for systemic administration may include at least one of diluents, lubricants, binders, disintegrants, colorants, flavors, sweeteners, antioxidants, preservatives, glidants, solvents, suspending agents, wetting agents, surfactants, combinations thereof, and others. All carriers are optional in the compositions.

[0099] Suitable diluents include sugars such as glucose, lactose, dextrose, and sucrose; diols such as propylene glycol; calcium carbonate; sodium carbonate; sugar alcohols, such as glycerin; mannitol; and sorbitol. The amount of diluent(s) in a systemic or topical composition is typically about 50 to about 90%.

[0100] Suitable lubricants include silica, talc, stearic acid and its magnesium salts and calcium salts, calcium sulfate; and liquid lubricants such as polyethylene glycol and vegetable oils such as peanut oil, cottonseed oil, sesame oil,

olive oil, corn oil, and oil of *theobroma*. The amount of lubricant(s) in a systemic or topical composition is typically about 5 to about 10%.

[0101] Suitable binders include polyvinyl pyrrolidone; magnesium aluminum silicate; starches such as corn starch and potato starch; gelatin; tragacanth; and cellulose and its derivatives, such as sodium carboxymethylcellulose, ethyl cellulose, methylcellulose, microcrystalline cellulose, and sodium carboxymethylcellulose. The amount of binder(s) in a systemic composition is typically about 5 to about 50%.

[0102] Suitable disintegrants include agar, alginic acid and the sodium salt thereof, effervescent mixtures, croscarmellose, crospovidone, sodium carboxymethyl starch, sodium starch glycolate, clays, and ion exchange resins. The amount of disintegrant(s) in a systemic or topical composition is typically about 0.1 to about 10%.

[0103] Suitable colorants include a colorant such as an FD&C dye. When used, the amount of colorant in a systemic or topical composition is typically about 0.005 to about 0.1%.

[0104] Suitable flavors include menthol, peppermint, and fruit flavors. The amount of flavor(s), when used, in a systemic or topical composition is typically about 0.1 to about 1.0%.

[0105] Suitable sweeteners include aspartame and saccharin. The amount of sweetener(s) in a systemic or topical composition is typically about 0.001 to about 1%.

[0106] Suitable antioxidants include butylated hydroxyanisole ("BHA"), butylated hydroxytoluene ("BHT"), and vitamin E. The amount of antioxidant(s) in a systemic or topical composition is typically about 0.1 to about 5%.

[0107] Suitable preservatives include benzalkonium chloride, methyl paraben and sodium benzoate. The amount of preservative(s) in a systemic or topical composition is typically about 0.01 to about 5%.

[0108] Suitable glidants include silicon dioxide. The amount of glidant(s) in a systemic or topical composition is typically about 1 to about 5%.

[0109] Suitable solvents include water, isotonic saline, ethyl oleate, glycerine, hydroxylated castor oils, alcohols such as ethanol, and phosphate buffer solutions. The amount of solvent(s) in a systemic or topical composition is typically from about 0 to about 100%.

[0110] Suitable suspending agents include AVICEL RC-591 (from FMC Corporation of Philadelphia, Pa.) and sodium alginate. The amount of suspending agent(s) in a systemic or topical composition is typically about 1 to about 8%.

[0111] Suitable surfactants include lecithin, Polysorbate 80, and sodium lauryl sulfate, and the TWEENS from Atlas Powder Company of Wilmington, Del. Suitable surfactants include those disclosed in the C.T.F.A. Cosmetic Ingredient Handbook, 1992, pp. 587-592; Remington's Pharmaceutical Sciences, 15th Ed. 1975, pp. 335-337; and McCutcheon's Volume 1, Emulsifiers & Detergents, 1994, North American Edition, pp. 236-239. The amount of surfactant(s) in the systemic or topical composition is typically about 0.1% to about 5%.

[0112] Although the amounts of components in the systemic compositions may vary depending on the type of systemic composition prepared, in general, systemic compositions include 0.01% to 50% of active agents and 50% to 99.99% of one or more carriers. Compositions for parenteral administration typically include 0.1% to 10% of actives and 90% to 99.9% of a carrier including a diluent and a solvent.

[0113] Compositions for oral administration can have various dosage forms. For example, solid forms include tablets, capsules, granules, and bulk powders. These oral dosage forms include a safe and effective amount, usually at least about 5%, and more particularly from about 25% to about 50% of actives. The oral dosage compositions include about 50% to about 95% of carriers, and more particularly, from about 50% to about 75%.

[0114] Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed. Tablets typically include an active component, and a carrier comprising ingredients selected from diluents, lubricants, binders, disintegrants, colorants, flavors, sweeteners, glidants, and combinations thereof. Specific diluents include calcium carbonate, sodium carbonate, mannitol, lactose and cellulose. Specific binders include starch, gelatin, and sucrose. Specific disintegrants include alginic acid and croscarmellose. Specific lubricants include magnesium stearate, stearic acid, and talc. Specific colorants are the FD&C dyes, which can be added for appearance. Chewable tablets preferably contain sweeteners such as aspartame and saccharin, or flavors such as menthol, peppermint, fruit flavors, or a combination thereof.

[0115] Capsules (including implants, time release and sustained release formulations) typically include an active compound, and a carrier including one or more diluents disclosed above in a capsule comprising gelatin. Granules typically comprise a disclosed compound, and preferably glidants such as silicon dioxide to improve flow characteristics. Implants can be of the biodegradable or the non-biodegradable type.

[0116] The selection of ingredients in the carrier for oral compositions depends on secondary considerations like taste, cost, and shelf stability, which are not critical for the purposes of this invention.

[0117] Solid compositions may be coated by conventional methods, typically with pH or time-dependent coatings, such that a disclosed compound is released in the gastrointestinal tract in the vicinity of the desired application, or at various points and times to extend the desired action. The coatings typically include one or more components selected from the group consisting of cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methylcellulose phthalate, ethyl cellulose, EUDRAGIT coatings (available from Rohm & Haas G.M.B.H. of Darmstadt, Germany), waxes and shellac.

[0118] Compositions for oral administration can have liquid forms. For example, suitable liquid forms include aqueous solutions, emulsions, suspensions, solutions reconstituted from non-effervescent granules, suspensions reconstituted from non-effervescent granules, effervescent preparations reconstituted from effervescent granules, elixirs, tinctures, syrups, and the like. Liquid orally administered compositions typically include a disclosed compound and a carrier, namely, a carrier selected from diluents, colorants, flavors, sweeteners, preservatives, solvents, suspending agents, and surfactants. Peroral liquid compositions preferably include one or more ingredients selected from colorants, flavors, and sweeteners.

[0119] Other compositions useful for attaining systemic delivery of the subject compounds include sublingual, buccal and nasal dosage forms. Such compositions typically

include one or more of soluble filler substances such as diluents including sucrose, sorbitol and mannitol; and binders such as acacia, microcrystalline cellulose, carboxymethyl cellulose, and hydroxypropyl methylcellulose. Such compositions may further include lubricants, colorants, flavors, sweeteners, antioxidants, and glidants.

[0120] The disclosed composition can be topically administered. Topical compositions that can be applied locally to the skin may be in any form including solids, solutions, oils, creams, ointments, gels, lotions, shampoos, leave-on and rinse-out hair conditioners, milks, cleansers, moisturizers, sprays, skin patches, and the like. Topical compositions may include: a disclosed glycan-remodeled glycoproteins (e.g., a glycan-remodeled lysosomal enzyme) and a carrier. The carrier of the topical composition preferably aids penetration of the compounds into the skin. The carrier may further include one or more optional components.

[0121] The amount of the carrier employed in conjunction with a disclosed glycan-remodeled glycoproteins (e.g., a glycan-remodeled lysosomal enzyme) is sufficient to provide a practical quantity of composition for administration per unit dose of the medicament. Techniques and compositions for making dosage forms useful in the present methods are described in the following references: Modern Pharmaceutics, Chapters 9 and 10, Banker & Rhodes, eds. (1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms, 2nd Ed., (1976).

[0122] A carrier may include a single ingredient or a combination of two or more ingredients. In the topical compositions, the carrier includes a topical carrier. Suitable topical carriers include one or more ingredients selected from phosphate buffered saline, isotonic water, deionized water, monofunctional alcohols, symmetrical alcohols, aloe vera gel, allantoin, glycerin, vitamin A and E oils, mineral oil, propylene glycol, PPG-2 myristyl propionate, dimethyl isosorbide, castor oil, combinations thereof, and the like. More particularly, carriers for skin applications include propylene glycol, dimethyl isosorbide, and water, and even more particularly, phosphate buffered saline, isotonic water, deionized water, monofunctional alcohols, and symmetrical alcohols.

[0123] The carrier of a topical composition may further include one or more ingredients selected from emollients, propellants, solvents, humectants, thickeners, powders, fragrances, pigments, and preservatives, all of which are optional.

[0124] Suitable emollients include stearyl alcohol, glyceryl monoricinoleate, glyceryl monostearate, propane-1,2-diol, butane-1,3-diol, mink oil, cetyl alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, isocetyl stearate, oleyl alcohol, isopropyl laurate, hexyl laurate, decyl oleate, octadecan-2-ol, isocetyl alcohol, cetyl palmitate, di-n-butyl sebacate, isopropyl myristate, isopropyl palmitate, isopropyl stearate, butyl stearate, polyethylene glycol, triethylene glycol, lanolin, sesame oil, coconut oil, arachis oil, castor oil, acetylated lanolin alcohols, petroleum, mineral oil, butyl myristate, isostearic acid, palmitic acid, isopropyl linoleate, lauryl lactate, myristyl lactate, decyl oleate, myristyl myristate, and combinations thereof. Specific emollients for skin include stearyl alcohol and polydimethylsiloxane. The amount of emollient(s) in a skin-based topical composition is typically about 5% to about 95%.

[0125] Suitable propellants include propane, butane, isobutane, dimethyl ether, carbon dioxide, nitrous oxide, and combinations thereof. The amount of propellant(s) in a topical composition is typically about 0% to about 95%.

[0126] Suitable solvents include water, ethyl alcohol, methylene chloride, isopropanol, castor oil, ethylene glycol monoethyl ether, diethylene glycol monobutyl ether, diethylene glycol monoethyl ether, dimethylsulfoxide, dimethyl formamide, tetrahydrofuran, and combinations thereof. Specific solvents include ethyl alcohol and homotopic alcohols. The amount of solvent(s) in a topical composition is typically about 0% to about 95%.

[0127] Suitable humectants include glycerin, sorbitol, sodium 2-pyrrolidone-5-carboxylate, soluble collagen, dibutyl phthalate, gelatin, and combinations thereof. Specific humectants include glycerin. The amount of humectant (s) in a topical composition is typically 0% to 95%.

[0128] The amount of thickener(s) in a topical composition is typically about 0% to about 95%.

[0129] Suitable powders include beta-cyclodextrins, hydroxypropyl cyclodextrins, chalk, talc, fullers earth, kaolin, starch, gums, colloidal silicon dioxide, sodium polyacrylate, tetra alkyl ammonium smectites, trialkyl aryl ammonium smectites, chemically-modified magnesium aluminum silicate, organically-modified Montmorillonite clay, hydrated aluminum silicate, fumed silica, carboxyvinyl polymer, sodium carboxymethyl cellulose, ethylene glycol monostearate, and combinations thereof. The amount of powder(s) in a topical composition is typically 0% to 95%.

[0130] Suitable pH adjusting additives include HCl or NaOH in amounts sufficient to adjust the pH of a topical pharmaceutical composition.

[0131] In some embodiments, the present pharmaceutical composition is suitable for intravenous administration. For example, the composition may be supplied (e.g., in a vial) as a sterile, nonpyrogenic, lyophilized cake or powder for reconstitution with sterile water for injection. The composition may include the glycan-remodeled glycoproteins (e.g., glycan-remodeled lysosomal enzyme) (e.g., 10-50 mg), mannitol (e.g., 100-500 mg), polysorbate 80 (e.g., 0.1-1 mg), sodium phosphate dibasic heptahydrate (e.g., 5-20 mg), sodium phosphate monobasic monohydrate (e.g., 10-50 mg). The composition may be reconstituted into an injectable solution.

Method of Pharmaceutical Use

[0132] The glycan-remodeled glycoproteins (e.g., glycan-remodeled lysosomal enzyme) may be used for treating a disease. The disease may be associated with, or caused by, a deficiency of the glycoprotein, for example, an enzyme deficiency.

[0133] Specifically, diseases which are caused by the deficiency in a single protein, e.g., single glycoprotein. For example, most lysosomal storage diseases are caused by a deficiency or dysregulation of a single lysosomal enzyme. Thus, in one embodiment, the present disclosure provides methods of treating a lysosomal storage disease comprising administering a glycan-remodeled lysosomal enzyme as described herein. The glycan-remodeled lysosomal enzyme comprises an M6P binding moiety that is capable of binding to cation-independent mannose 6-phosphate receptor (CI-MPR) on the surface of cells. This allows for the targeting and delivery of the glycan-remodeled lysosomal enzymes to the lysosome of the cell via the CI-MPR.

[0134] In some embodiment, the disclosure provides a method of treating a lysosomal storage disease, the method comprising administering an effective amount of a glycan-remodeled lysosomal enzyme comprising an M6P moiety capable of binding to CI-MPR and treating the lysosomal storage disease.

[0135] Suitable lysosomal storage diseases are listed in Table 1, along with the enzyme glycoprotein that is able to be remodeled by the methods described herein for use in treating the lysosomal storage disease.

[0136] In one example, and as described in the Examples below, the lysosomal storage disease is Pompe disease, and the methods described herein are used to remodel α-glucosidase (rhGAA). rhGAA is used as a model therapeutic enzyme to demonstrate the efficiency of the glycan remodeling method. But this method should be applicable for the glycan remodeling of all the lysosomal enzymes for the site-selective introduction of the high-affinity M6P ligands for improving the therapeutic efficacy of those therapeutic enzymes used in enzymatic replacement therapy (ERT), as shown in Table 2.42

TABLE 2

Examples of LSDs and Corresponding Lysosomal Hydrolases

Lysosomal Storage Disorder	Defective Enzyme	Protein Sequence
Fabry	α-Galactosidase A	UniProtKB - P06280 (SEQ ID NO: 4)
Farber	Acid ceramidase	UniProtKB - Q13510 (SEQ ID NO: 5)
Fucosidosis	Acid α-L-fucosidase	UniProtKB - P04066 (SEQ ID NO: 6)
Gaucher types 1, 2, and 3	Acid β-glucosidase	UniProtKB - P04062 (SEQ ID NO: 7)
GM1 gangliosidosis	Acid β-galactosidase	UniProtKB - P16278 (SEQ ID NO: 8)
Hunter (Mucopolysaccharidosis (MPS) II)	Iduronate-2-sulfatase	UniProtKB - P22304 (SEQ ID NO: 9)
Hurler-Schele, Hurler, Schele (MPS I)	α-L-Iduronidase	UniProtKB - P35475 (SEQ ID NO: 10)
Krabbe	Galactocerebrosidase	UniProtKB - P54803 (SEQ ID NO: 11)
α-Mannosidosis	Acid α-mannosidase	UniProtKB - O00754 (SEQ ID NO: 12)
β-Mannosidosis	Acid β-mannosidase	UniProtKB - O00462 (SEQ ID NO: 13)
Maroteaux-Larny (MPS VI)	Arylsulfatase B	UniProtKB - P15289 (SEQ ID NO: 14)
Metachromatic leukodystrophy	Arylsulfatase A	UniProtKB - P15289 (SEQ ID NO: 15)
Morquio A (MPS IV)	N-Acetylgalactosamine-6-sulfate sulfatase (N-acetylgalactosamine-6-sulfatase) (galactose-6-sulfatase)	UniProtKB - P34059 (SEQ ID NO: 16)
Morquio B (MPS IV)	Acid β-galactosidase	UniProtKB - P16278 (SEQ ID NO: 8)
Niemann-Pick A and B	Acid sphingomyelinase	UniProtKB - P17405 (SEQ ID NO: 17)
Pompe	Acid α-glucosidase (α-glucosidase)	UniProtKB - P10253 (SEQ ID NO: 18)
Sandhoff	β-Hexosaminidase B	UniProtKB - P07686 (SEQ ID NO: 19)
Sanfilippo A (MPS III)	Heparan N-sulfatase	UniProtKB - Q68CP4 (SEQ ID NO: 20)
Sanfilippo B (MPS III)	α-N-Acetylglucosaminidase	UniProtKB - P54802 (SEQ ID NO: 21)
Sanfilippo C (MPS III)	Acetyl-CoA: α-glucosaminide N-acetyltransferase	UniProtKB - Q68CP4 (SEQ ID NO: 22)
Sanfilippo D (MPS III)	N-Acetylglucosaminide-6-sulfate sulfatase	UniProtKB - P15586 (SEQ ID NO: 23)
Schindler-Kanzaki	α-N-acetylgalactosaminidase	UniProtKB - P17050 (SEQ ID NO: 24)
Sialidosis	Sialidase	UniProtKB - Q99519 (SEQ ID NO: 25)
Sly (MPS VII)	β-Glucuronidase	UniProtKB - P08236 (SEQ ID NO: 26)
Tay-Sachs	β-Hexosaminidase A	UniProtKB - P07686 (SEQ ID NO: 27)

Protein sequences are incorporated by reference in their entirety.

[0137] For example, the disease may be Pompe disease, which is caused by a deficiency of a lysosomal enzyme, such as acid α-glucosidase (GAA). Pompe disease is an inherited disorder resulting from the buildup of a complex sugar called glycogen in the body's cells resulting in the accumulation of glycogen in certain organs and tissues, especially muscles, which impairs their ability to function normally. Mutations within the GAA gene cause Pompe disease as the GAA gene provides instructions for producing an enzyme called acid α-glucosidase (also known as acid maltase). This enzyme is active in lysosomes which serve as recycling centers within cells. The enzyme normally breaks down glycogen in lysosomes into a simpler sugar called glucose, which is the main energy source for most cells.

[0138] In one aspect, the present disclosure provides a method of treating Pompe disease in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of the glycan-remodeled lysosomal enzyme as described herein.

[0139] The subject may be a mammal, in particular a human.

[0140] For purposes of the present invention, "treating" or "treatment" describes the management and care of a subject for combating a disease, condition, or disorder. Treating includes the administration of an active agent (such as a therapeutic enzyme or a pharmaceutical composition as disclosed herein) for preventing the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder. As used herein, and as well understood in the art, "treatment" or "treating" is also an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of disease, stabilized (i.e., not worsening) state of disease, preventing the spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Further, any of the treatment methods or uses described herein can be formulated alone or for contemporaneous administration with other agents or therapies.

[0141] As used herein, the phrase "effective amount" or "therapeutically effective amount" or a "sufficient amount" of a glycoprotein or composition of the present application is a quantity sufficient to, when administered to the subject, effect beneficial or desired results, including clinical results, and, as such, an "effective amount" or synonym thereto depends upon the context in which it is being applied. The amount given should be varied depending upon various factors, such as the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, weight) or host being treated, and the like, but can nevertheless be routinely determined by one skilled in the art. Also, as used herein, a "therapeutically effective amount" of the present disclosure is an amount, which results in a beneficial or desired result in a subject as compared to a control. As defined herein, a therapeutically effective amount of a compound of the present disclosure may be readily determined by one skilled in the art by routine methods known in the art. Dosage regime may be adjusted to provide the optimum therapeutic response.

[0142] The glycan-remodeled lysosomal enzyme may be administered, for example, in a pharmaceutical composition as described herein. In some embodiments, the glycan-remodeled lysosomal enzyme is administered intravenously. In some embodiments, the glycan-remodeled lysosomal enzyme is administered orally.

[0143] In particular embodiments, the glycan-remodeled lysosomal enzyme is a glycan-remodeled acid-glucosidase (α-glucosidase). The glycan-remodeled lysosomal enzyme may be administered, for example, by intravenous injection or intravenous infusion. The administration may be daily, weekly, biweekly, or monthly. The administration dosage may be about 1 mg/kg to about 100 mg/kg body weight, such as about 1 mg/kg to about 50 mg/kg or about 1 mg/kg to about 25 mg/kg body weight. The dosage may be, for example, about 1 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 50 mg/kg, or about 75 mg/kg body weight. For example, the glycan-remodeled lysosomal enzyme may be administered by intravenous infusion every two weeks at a dosage of about 20 mg/kg body weight.

[0144] As used herein, "subject" or "patient" refers to mammals and non-mammals. "Mammals" means any member of the class Mammalia including, but not limited to, humans, non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, and swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice, and guinea pigs; and the like. Examples of non-mammals include, but are not limited to, birds, and the like. The term "subject" does not denote a particular age or sex. In one specific embodiment, a subject is a mammal, preferably a human. In some embodiments, the subject is a human suffering from a disease or disorder needing therapeutic treatment. Suitable subjects may have a disease or disorder, specifically a disease or disorder that can be treated by a protein or enzyme of interest, as described herein.

[0145] As used herein, the terms "administering" and "administration" refer to any method of providing a pharmaceutical preparation or composition to a subject. Such methods are well known to those skilled in the art and include, but are not limited to, oral administration, transdermal administration, administration by inhalation, nasal administration, topical administration, intravaginal administration, ophthalmic administration, intraaural administration, intracerebral administration, rectal administration, sublingual administration, buccal administration, and parenteral administration, including injectable such as intravenous administration, intra-arterial administration, intramuscular administration, intradermal administration, intrathecal administration and subcutaneous administration. Administration can be continuous or intermittent. In various aspects, a preparation can be administered therapeutically; that is, administered to treat an existing disease or condition. In some embodiments, the administration is intravenous administration.

[0146] It should be apparent to those skilled in the art that many additional modifications beside those already described are possible without departing from the inventive concepts. In interpreting this disclosure, all terms should be interpreted in the broadest possible manner consistent with the context. Variations of the term "comprising" should be

interpreted as referring to elements, components, or steps in a non-exclusive manner, so the referenced elements, components, or steps may be combined with other elements, components, or steps that are not expressly referenced. Embodiments referenced as "comprising" certain elements are also contemplated as "consisting essentially of" and "consisting of" those elements. In places where ranges of values are given, this disclosure explicitly contemplates other combinations of the lower and upper limits of those ranges that are not explicitly recited. For example, recitation of a value between 1 and 10 or between 2 and 9 also contemplates a value between 1 and 9 or between 2 and 10. Ranges identified as being "between" two values are inclusive of the end-point values. For example, recitation of a value between 1 and 10 includes the values 1 and 10.

[0147] Aspects of the present disclosure that are described with respect to methods can be utilized in the context of the compositions of matter or kits discussed in this disclosure. Similarly, aspects of the present disclosure that are described with respect to compositions of matter can be utilized in the context of the methods and kits, and aspects of the present disclosure that are described with respect to kits can be utilized in the context of the methods and compositions of matter.

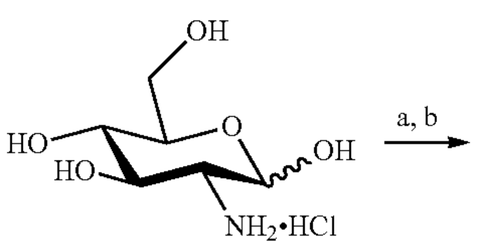
[0148] The scope of the disclosure will be more fully understood upon consideration of the following non-limiting examples.

EXAMPLES

Example 1: Chemical Synthesis of Truncated High-Mannose Type Phosphorylated N-Glycan Oxazolines

[0149] Following the previously reported synthesis of phosphorylated N-glycans,[18, 29, 30] selectively protected core disaccharide 15 was synthesized. Starting from D-glucosamine hydrochloride, 11 was prepared in two steps following the reported procedure.[32] Compound 11 was then treated with Ag_2CO_3 and BnBr to introduce a benzyl group to the anomeric position, followed by 3-O-benzylation with NaH and BnBr to yield 12 in 75% overall yield. The β-configuration was confirmed by the coupling constant of $J_{1,2}$=7.8 Hz. Upon regioselective ring-opening of the benzylidene group, 13 was obtained in 81% yield, which was coupled with the known compound 1433 to give the core disaccharide 15 in 69% yield. Next, regioselective ring-opening reaction furnished 16 with a free OH at C6 position, and after the conversion of the 2-azido group into the 2-acetamido group with AcSH,[30] the free OH was phosphorylated with dibenzyl N,N-diisopropylphosphoramidite, followed by oxidation with mCPBA[18] to give 18 in 90% yield. Global deprotection of the Bn and PMB groups via a two-step catalytic hydrogenolysis[30] gave the free disaccharide 19 in excellent yield. Finally, oxazoline formation was achieved in a single step by treatment with an excess amount of 2-chloro-1,3-dimethylimidazolinium chloride (DMC)[34] in water in the presence of Et_3N to afford the phosphorylated core disaccharide oxazoline 5 in 97% yield (Scheme 1).

Scheme 1. Synthesis of phosphorylated core disaccharide oxazoline 5.



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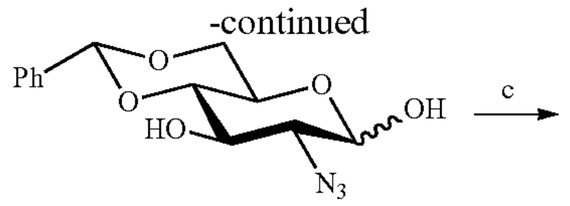

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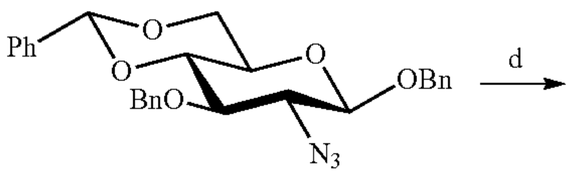

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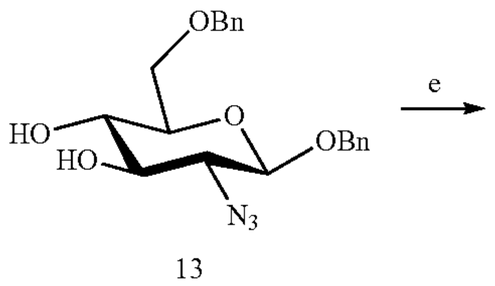

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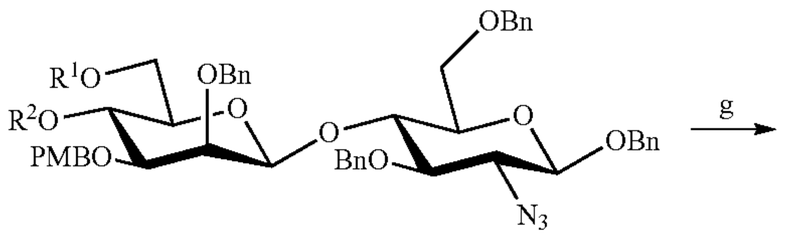

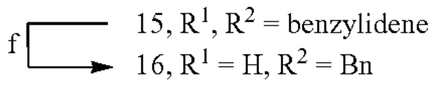

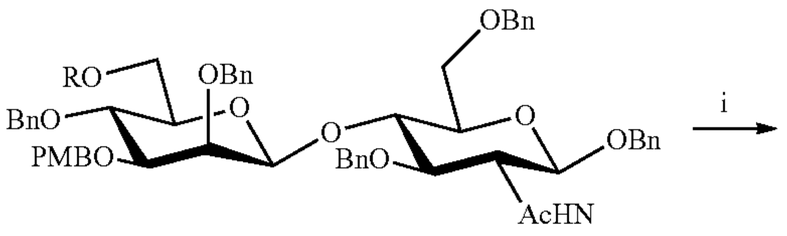

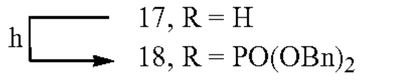

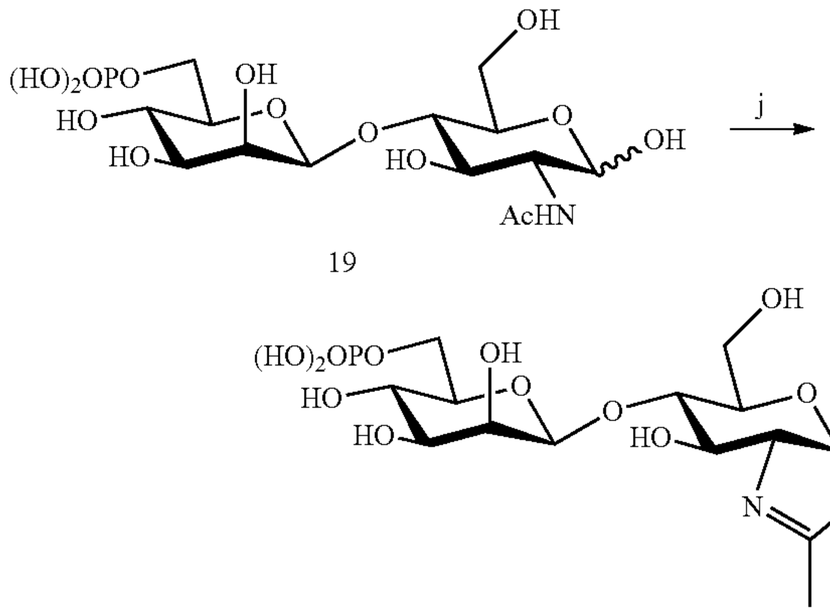

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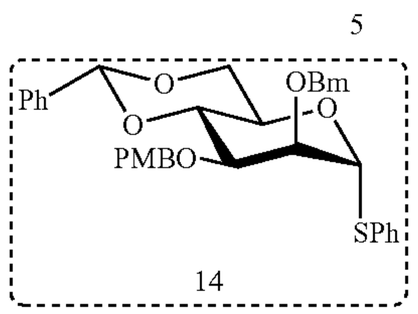

Reagents and conditions: a) TfN_3, K_2CO_3, $CuSO_4$, CH_2Cl_2/H_2O, 0° C.~RT; b) $PhCH(OMe)_2$, CSA, MeCN, 73% for 2 steps; c) BnBr, Ag_2CO_3, MeCN, 60° C., then BnBr, NaH, DMF, 0° C.~ RT, 75%; d) Et_3SiH, BF_3•OEt_2, DCM, 0° C., 81%; e) 14, BSP TTBP, Tf_2O, CH_2Cl_2, 4Å MS, -60° C., 69% f) BH_3•THF, Bu_2BOTf, CH_2Cl_2, 0° C., 91%; g) AcSH, pyridine/$CHCl_3$, RT, 84%; h) $(BnO)_2PNiPr_2$, tetrazole, 4Å MS, CH_2Cl_2, then mCPBA, -30° C., 90%; i) Pd/C, H_2, THF/MeOH, then $Pd(OH)_2$/C, H_2, MeOH/H_2O, 95%; j) DMC, Et_3N, H_2O, 0° C., 97%.

[0150] To determine the role of the mannosyl branches, trisaccharide oxazolines 6 and 7 were synthesized with a phosphate group at the α-1,6 or α-1,3-branch, respectively (Scheme 2). Deprotection of the 3-O-PMB in 15 gave 20 as an acceptor, then 16 and 20 were glycosylated with glycosyl donor 21[35] to give trisaccharides 23 and 24, respectively. A two-step manipulation was conducted to convert the benzoyl group to permanent benzyl group, giving 26 in 90% yield. However, the benzylation step failed to afford 25 even under harsh conditions probably due to high steric hindrance, thus the 2-O-Bn imidate 22[36] was used as the donor, which furnished 25 in 72% yield along with 15% of the β isomer. Next, upon the reduction of the azido group to the acetamido group followed by the selective deprotection of the TIPS group with TBAF, 29 and 30 were obtained in 85% and 87% yield, respectively, which were ready for phosphorylation. Finally, the phosphate group was introduced at the C6 position to give the fully protected derivatives 31 and 32 in 75% and 90% yield, respectively. Global deprotection via hydrogenolysis removed all the Bn, PMB and benzylidene groups simultaneously, giving free trisaccharides 33 and 34, which were converted into glycan oxazolines 6 and 7 respectively by reaction with DMC in a single step.

Scheme 2. Synthesis of monophosphorylated trisaccharide oxazolines 6 and 7.

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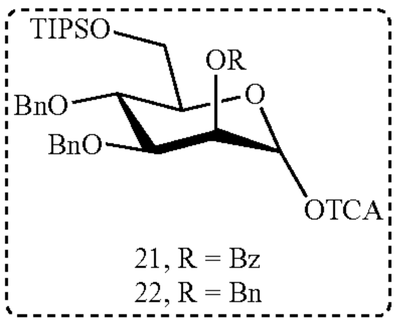
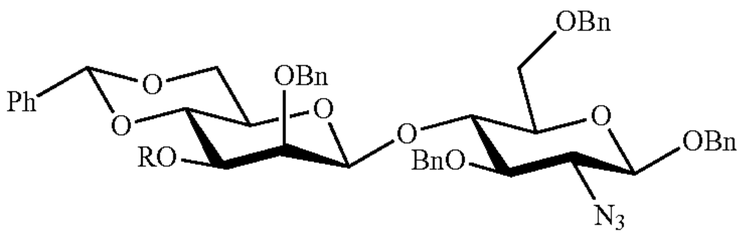
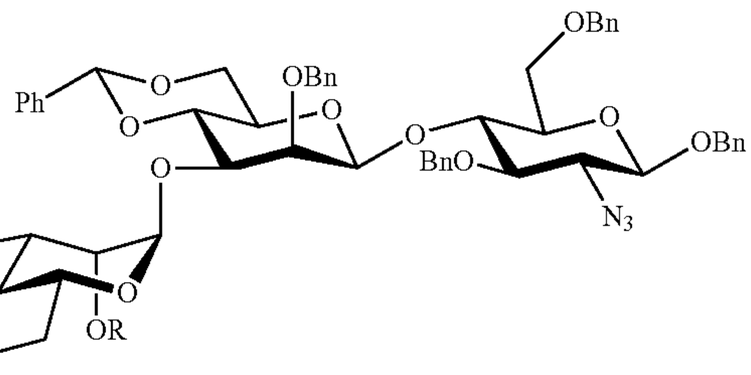


21, R = Bz
22, R = Bn



a: 15, R = PMB → 20, R = H

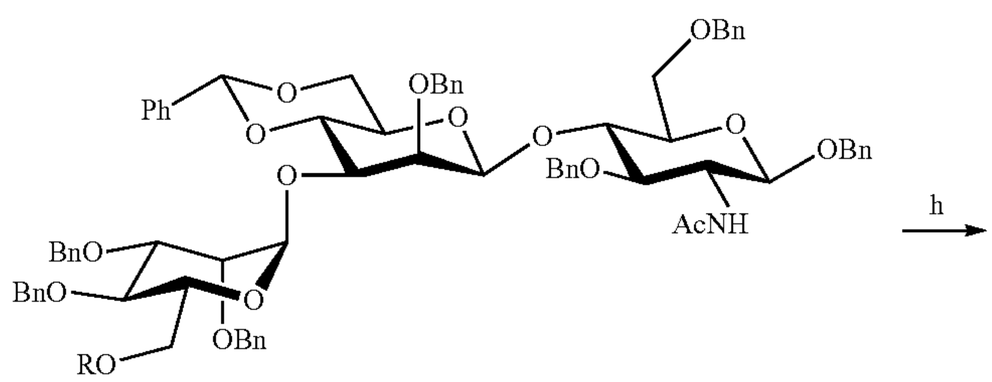
b



c, d: 24, R = Bz → 26 R = Bn

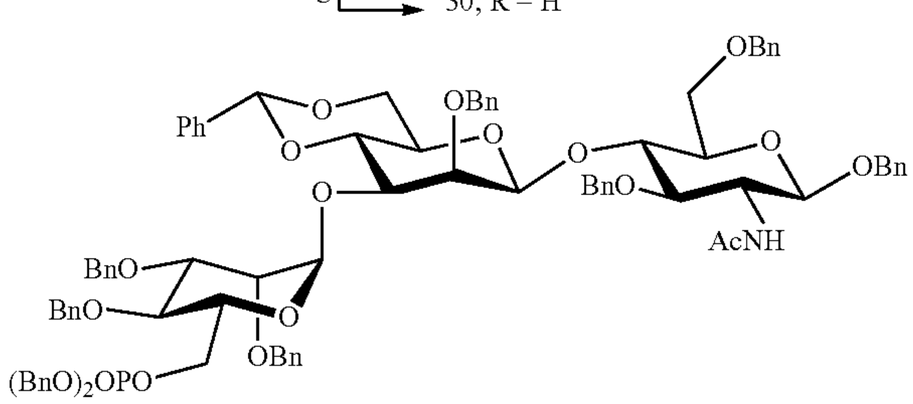
f

(?)



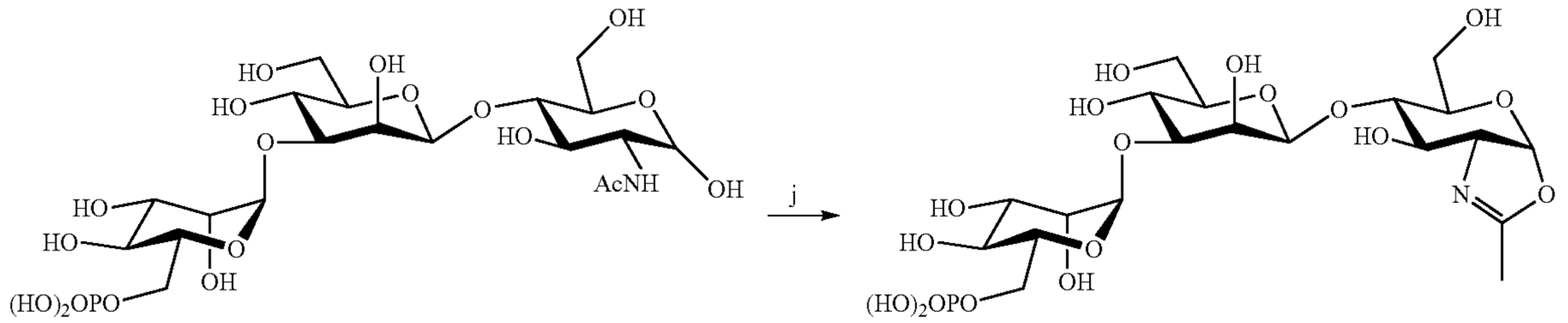
h

g: 28, R = TIPS → 30, R = H



i

32



34 7

Reagents and conditions: a) DDQ, CH_2Cl_2/H_2O, 0° C.~RT, 90%; b) 21, TMSOTf, 4Å MS, CH_2Cl_2, -30° C., 23, 94%, 24, 92%; c) CH_3ONa, MeOH, 50° C.; d) BnBr, NaH, DMF, 0° C.~RT, 26, 90% for 2 steps; e) 22, TMSOTf, 4Å MS, CH_2Cl_2, -30° C., 72%; f) AcSH, pyridine/$CHCl_3$, 60° C., 27, 89%, 28, 94%; g) TBAF, THF, RT, 29, 85%, 30, 87%; h) $(BnO)_2PNiPr_2$, tetrazole, 4Å MS, CH_2Cl_2, then mCPBA, -30° C., 31, 75%, 32, 90%; i) Pd/C, H_2, THF/MeOH, then $Pd(OH)_2/C$, H_2, MeOH/H_2O, 33, 96%, 34, 80%; j) DMC, Et_3N, H_2O 0° C., 6, quant., 7, 95%.

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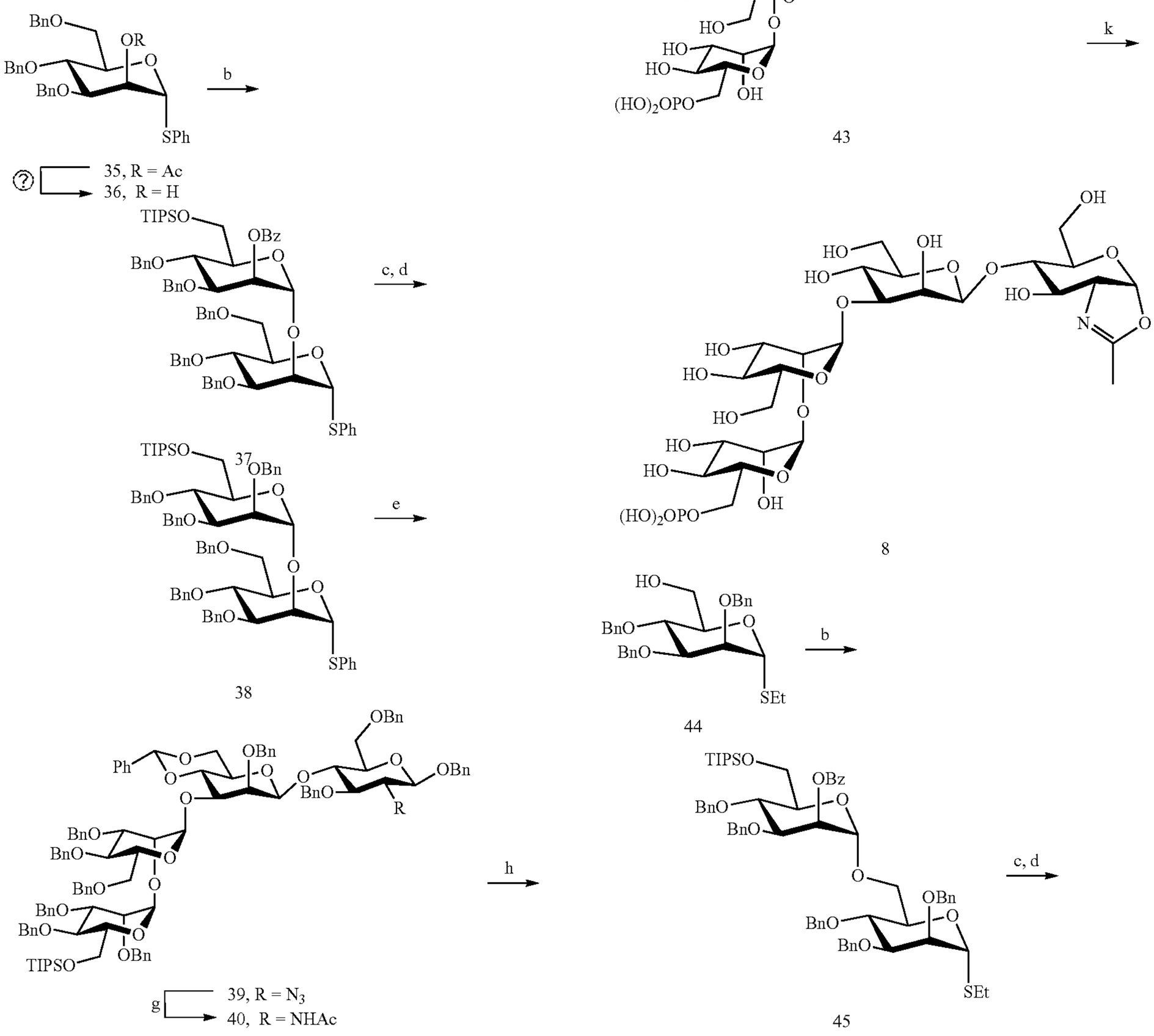
[0151] For the synthesis of tetrasaccharides with only α-1,3 or α-1,6-branch, the disaccharide 37 was first synthesized by coupling of donor 21 with the known acceptor 36.37 After the conversion of the benzoyl group to permanent benzyl group, the resulting disaccharide 38 was then used as the glycosyl donor for coupling with 20 under the promotion of NIS and TfOH to provide 39 in 74% yield, which was confirmed as the sole a isomer. After reduction of the azido group to acetamido group, followed by the selective deprotection of TIPS group, the C6 free OH was phosphorylated to give 42. Finally, the global deprotection and oxazoline formation provided the oxazoline 8 in good yield (Scheme 3). In parallel, the 1,6-linked disaccharide 45 was prepared by the coupling of donor 21 with 44,[38] which furnished the glycosyl donor 46 upon the conversion of the benzoyl group to benzyl group. The [2+2] coupling of 46 and 16 afforded 47 in 72% yield along with 17% of β isomer. Conversion of the azido group to acetamido group, followed by deprotection of TIPS and standard phosphorylation gave 50 in good yield. Finally, global deprotection of 50 followed by DMC treatment gave the oxazoline 9 (Scheme 3).

Scheme 3. Synthesis of monophosphorylated tetrasaccharide oxazolines 8 and 9.



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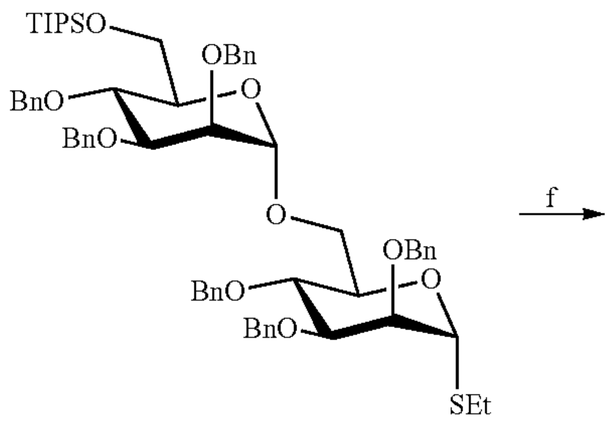
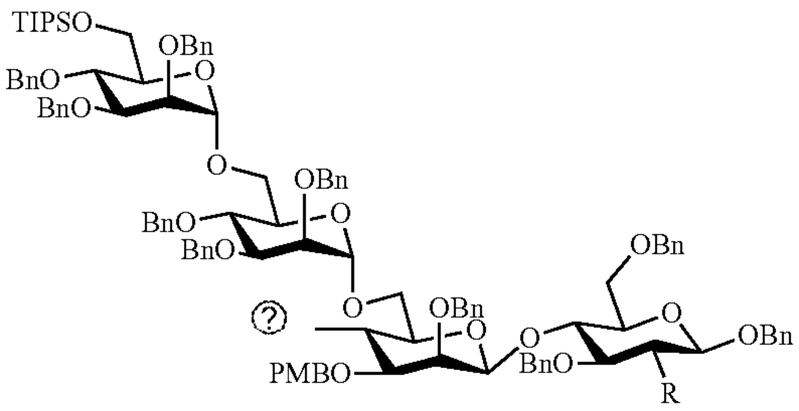
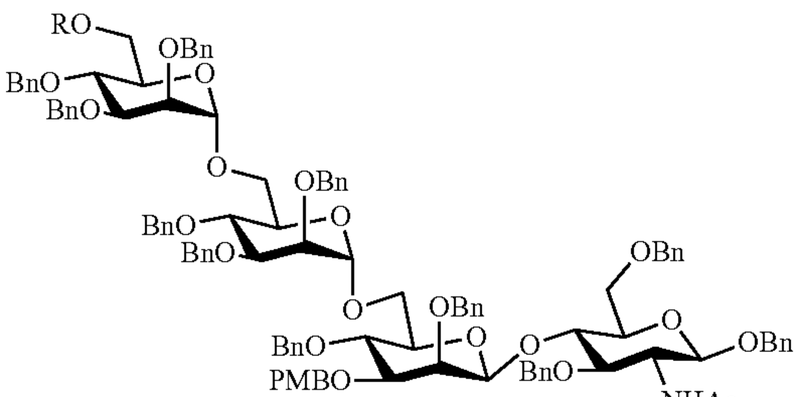


46



47, R = N_3

48, R = NHAc

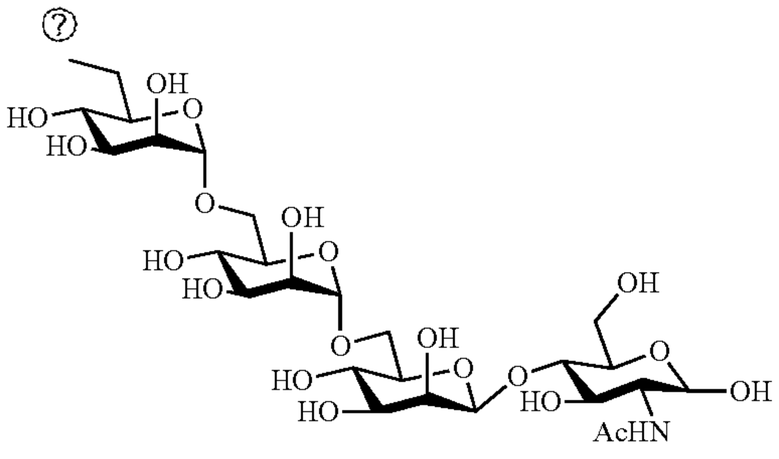


NHAc

i

48, R = N

50, R = $PO(OBn)_2$



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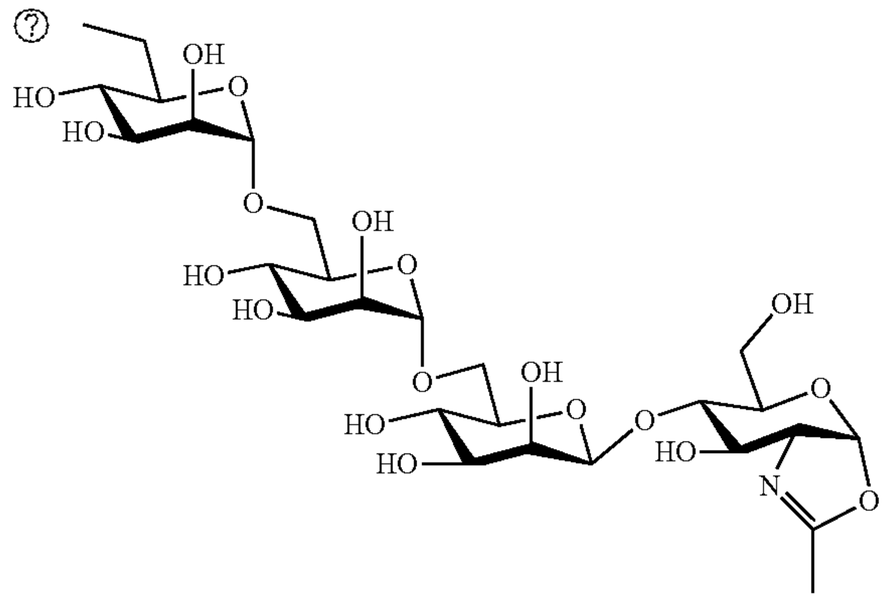

Reagents and conditions: a) CH_3ONa, $MeOH/CH_2Cl_2$, RT, 90%; b) 21, TMSOTf, 4Å MS, CH_2Cl_2, -30° C., 37, 96%, 45, 94%; c) CH_3ONa, MeOH, 50° C.; d) BnBr, NaH, DMF, 0- C.~RT, 38, 83% for 2 steps, 46, 89% for 2 steps; e) 20, NIS, TfOH, 4Å MS, CH_2Cl_2, -30° C., 74%; f) 16, NIS, AgOTf, 4Å MS, CH_2Cl_2/Et_2O, -40° C., 72%; g) AcSH, pyridine/$CHCl_3$, 60° C., 40, 86%, 48 85%; h) TBAF, THF, RT, 41, 83%, 49, 70%; i) $(BnO)_2PNiPr_2$, tetrazole, 4Å MS, CH_2Cl_2, then mCPBA, -30° C., 42, 70%, 50, 88%; j) Pd/C, H_2, THF/MeOH, then $Pd(OH)_2/C$, H_2, $MeOH/H_2O$, 43, 95%, 51, 91%; k) DMC, Et_3N, H_2O, 0° C., 8, 87%, 9, 90%.

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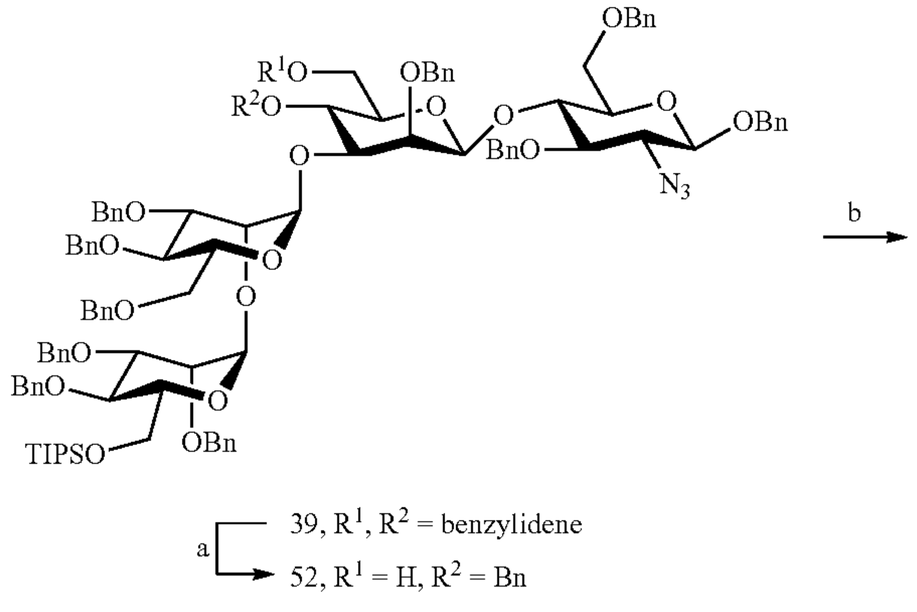
[0152] Previous studies have shown that not only the glycan structure determinants but also its optimal orientation is important for high-affinity binding with CI-MPR.[17, 39, 40] Considering the critical role of the Man3 core for retaining the glycan conformation, the pentasaccharide oxazoline 10 was designed and synthesized. Starting with tetrasaccharide 39, regioselective ring-opening reaction afforded 52 with a C6 free OH at the core mannosyl residue, then another mannosyl residue was installed at this position using glycosyl donor 3537 to give 53 in 80% yield. After the acetyl group was converted into benzyl group, oxazoline 10 was readily obtained as described for the synthesis of 5~9 by sequential reduction of azido group, deprotection of TIPS, phosphorylation, global deprotection and final oxazoline formation (Scheme 4).

Scheme 4.



39, R^1, R^2 = benzylidene

52, R^1 = H, R^2 = Bn

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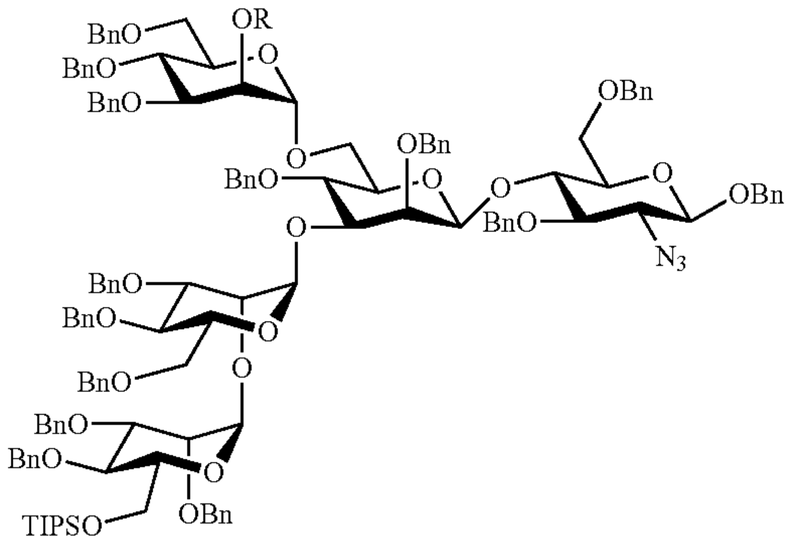

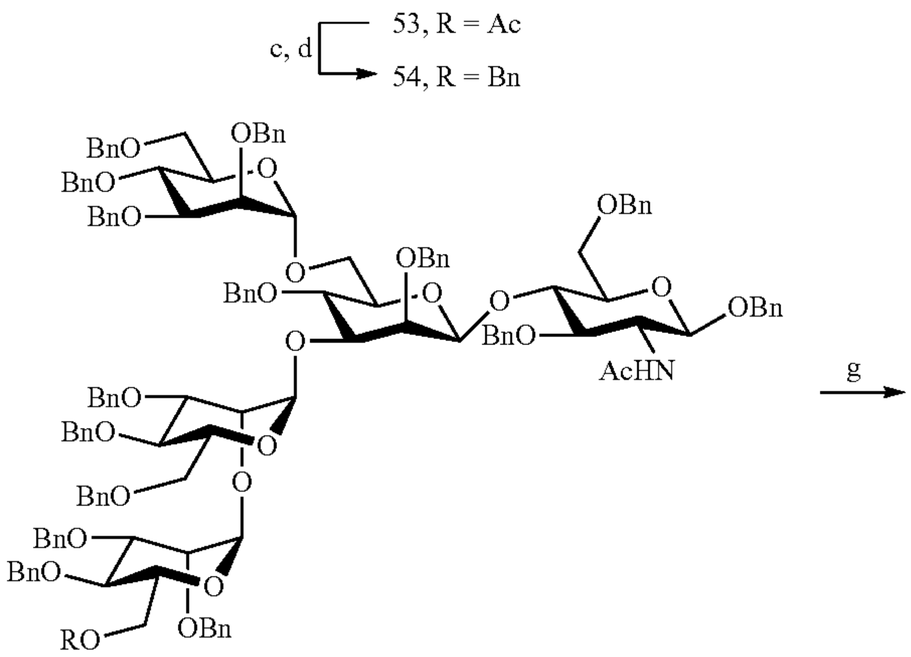

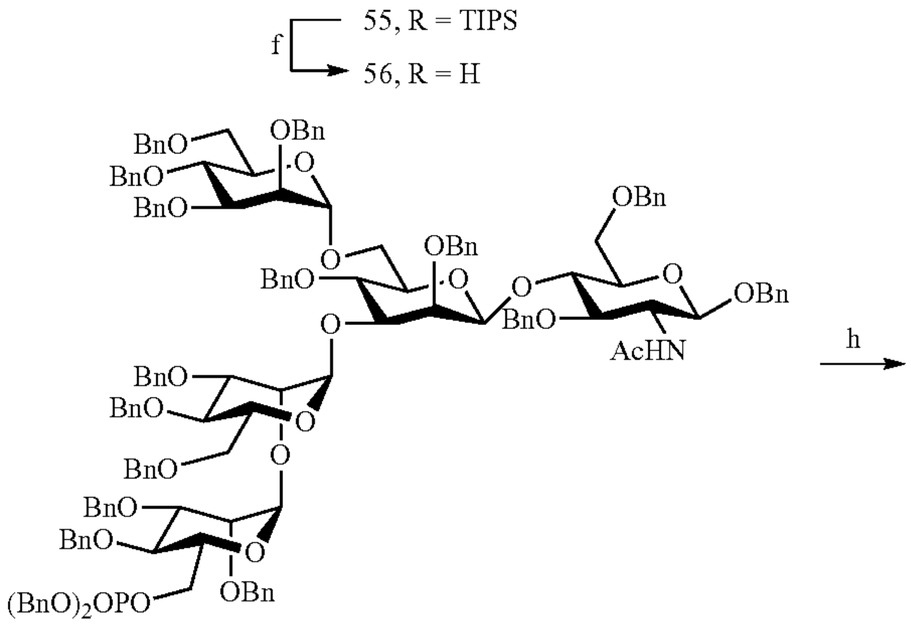

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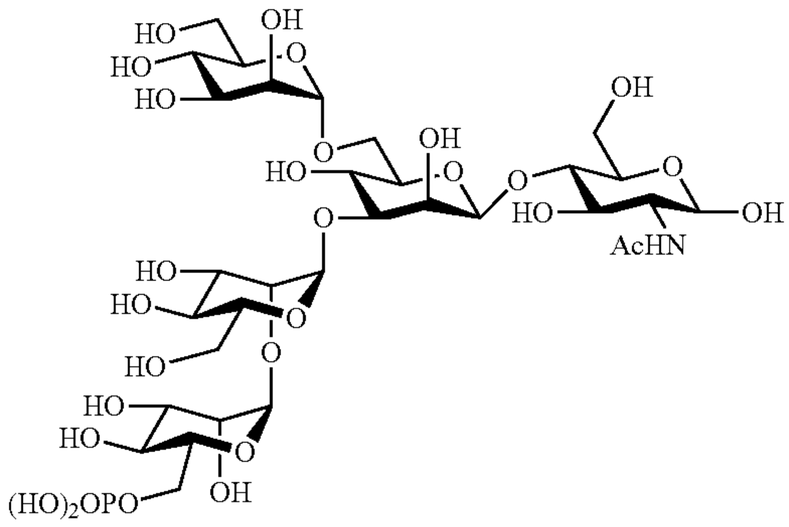

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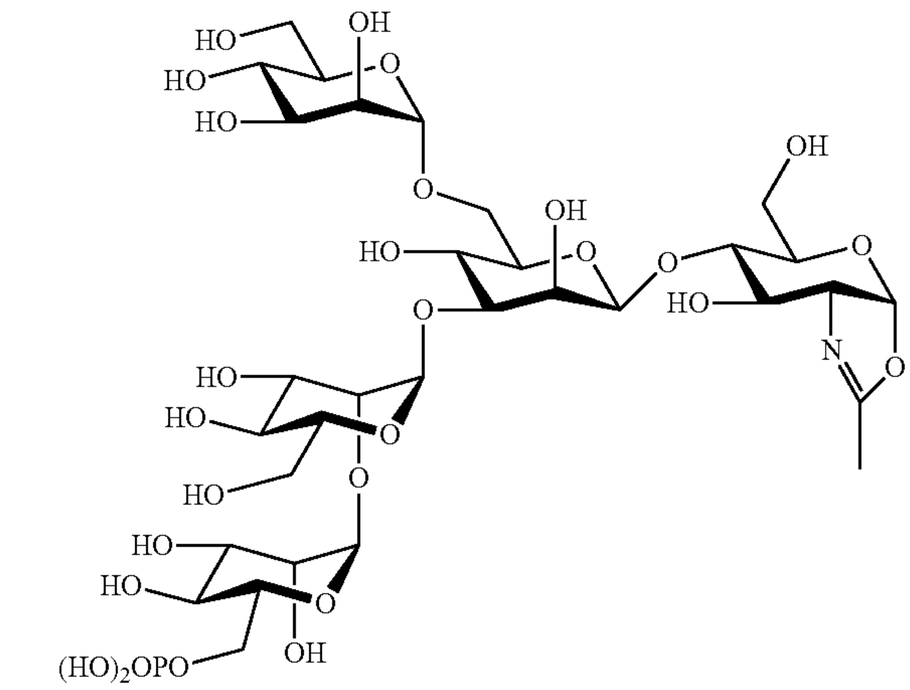


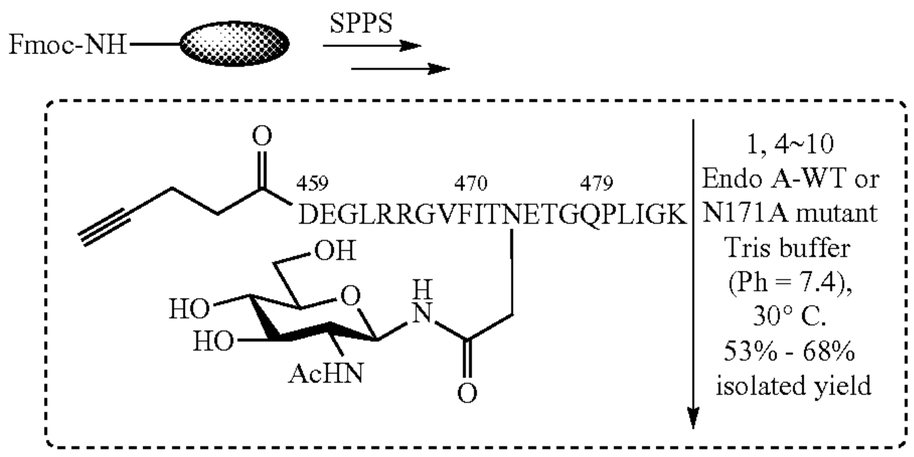
10

Synthesis of monophosphorylated pentasaccharide oxazline 10. Reagents and conditions: a) Et_3SiH, $PhBCl_2$, 4Å MS, CH_2Cl_2, -78° C., 79%; b) 35, NIS, TfOH, 4Å MS, CH_2Cl_2, -30° C., 80%; c) CH_3ONa, CH_2Cl_2/MeOH, RT; d) BnBr, NaH, DMF, 0° C. ~RT, 85% for 2 steps; e) AcSH, pyridine/$CHCl_3$, 60° C., 84%; f) TBAF, THF, RT, 90%; g) $(BnO)_2PNiPr_2$, tetrazole, 4Å MS, CH_2Cl_2, then mCPBA, -30° C., 90%; h) Pd/C, H_2, THF/MeOH then $Pd(OH)_2$/C, H_2, MeOH/H_2O, 77%; i) DMC, Et_3N, H_2O, 0° C., 93%.

Example 2: Evaluation of the Synthetic M6P-Glycan Oxazolines as Donor Substrates for Enzymatic Transglycosylation

[0153] The activities of the synthetic phosphorylated oxazolines as donor substrates for enzymatic transglycosylation were tested. For that purpose, a 21-mer glycopeptide (aa 459-479) derived from rhGAA was selected as a model sequence, in which the N470 residue was selected to install an M6P-glycan,[16] and the precursor GlcNAc-peptide was synthesized via automated solid-phase peptide synthesis (SPPS). Previous studies have shown that wild-type Endo-A, an endoglycosidase from *Arthrobacter protophormiae,*[41] is efficient for the transglycosylation of truncated phosphorylated Man3GlcNAc oxazoline to GlcNAc-peptides,[29, 30] but not suitable for transferring large natural M6P high-mannose N-glycan oxazolines, due to its rapid hydrolysis of both the oxazoline donors and the resulting transglycosylation products.[30] In this study, the truncated structures (4~10) acted as good substrates of wild-type Endo-A, and the resulting products, once formed, were barely hydrolyzed by the enzyme, affording the desired glycopeptides (59~65) in good isolated yields. The newly formed phosphorylated products were eluted later than the GlcNAc-peptide under the reverse-phase HPLC condition, and the identities of the products were confirmed with ESI-MS (FIG. **6**). Notably, the transglycosylation yields could be driven to 80%~90% if additional sugar oxazolines were added to the reaction. Finally, for the bis-phosphorylated ManGlcNAc oxazoline 1, Endo-A-N171A was used and the glycopeptide 66 was obtained in 53% yield after purification by HPLC (Scheme 5).

Scheme 5. Synthesis of GlcNAc-peptide derived from rhGAA. Reagents and conditions: GlcNAc-peptide (2.0 ~ 3.0 mg), oxazoline (4~6 eq) with Endo-A WT (for 59~65) or Endo-A N171A (for 66) was incubated in Tris buffer (100 mM, pH = 7.4) at 30° C. 59, 60%, 60, 66%, 61, 60%, 62, 66%, 63, 57%, 64, 68%, 65, 54%, 66, 53%.



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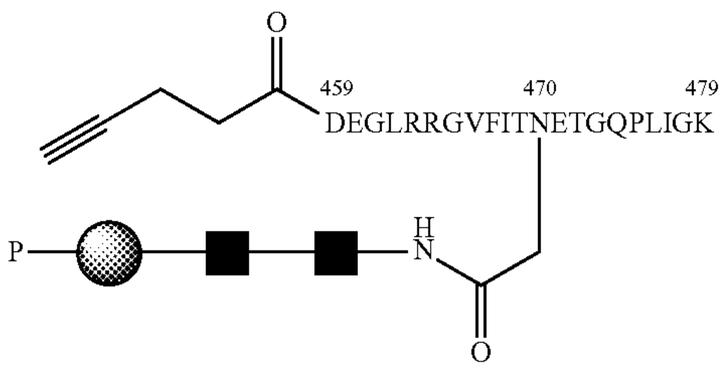

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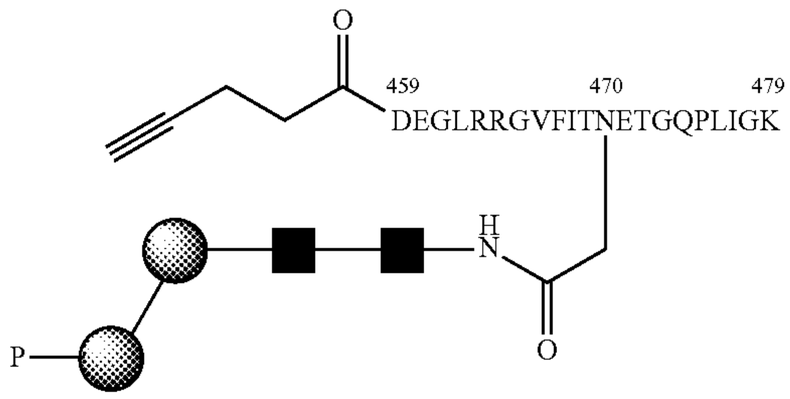

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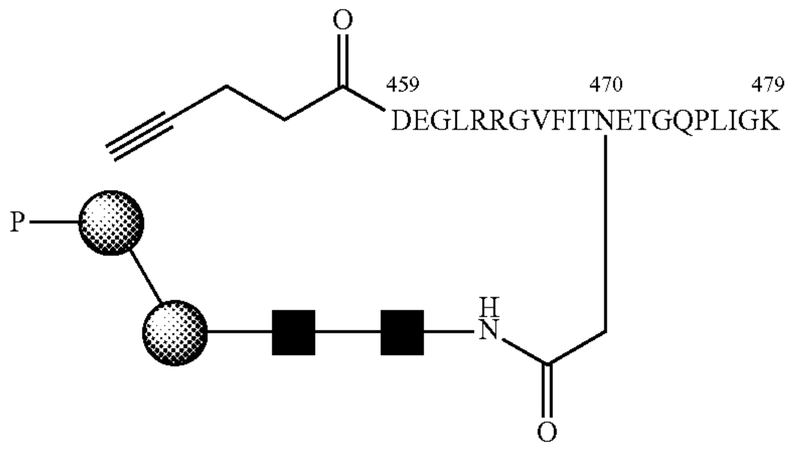

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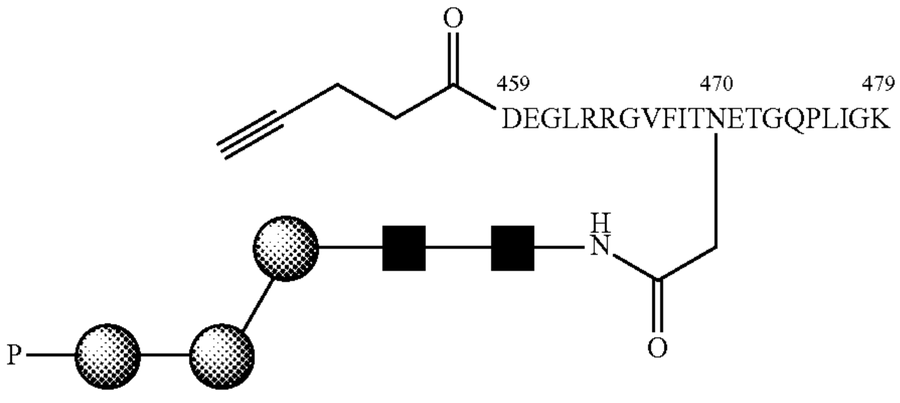

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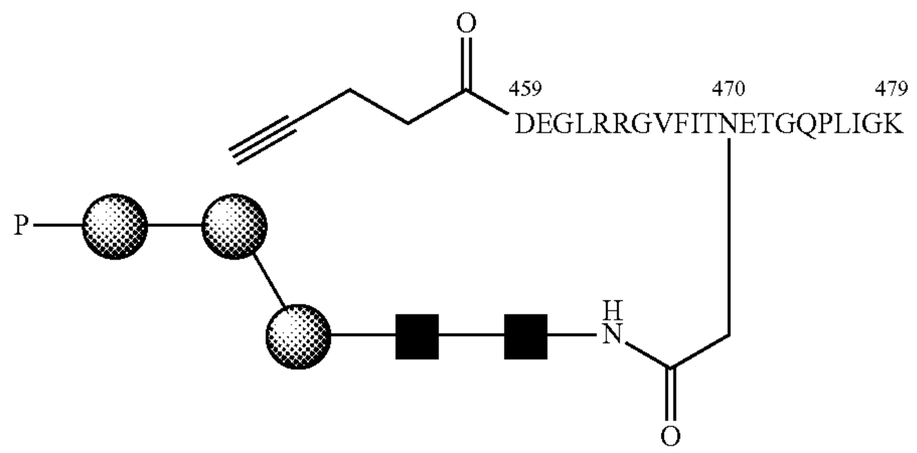

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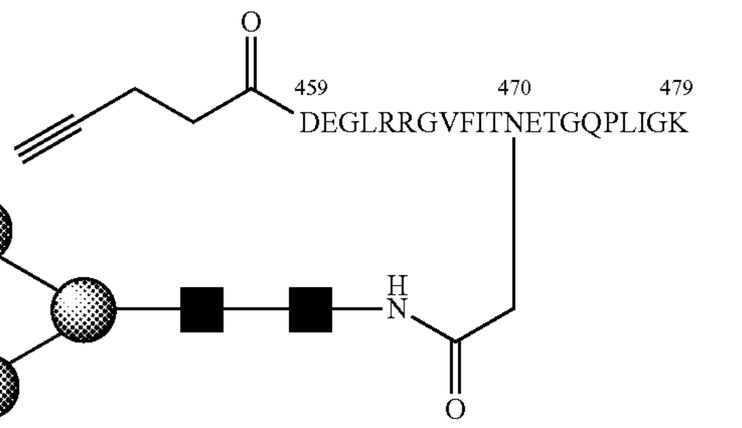

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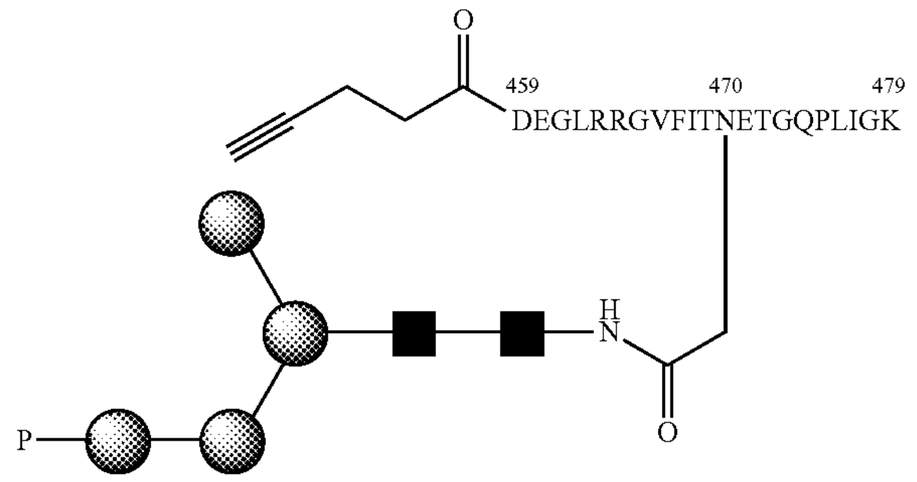

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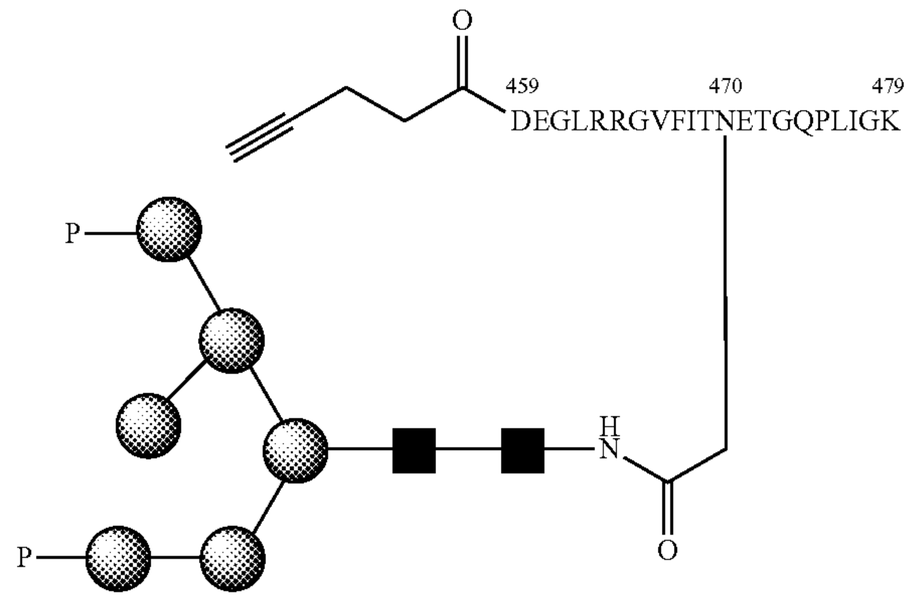


Example 3: Evaluation of the Affinity of the Synthetic M6P-Glycopeptides for the M6P Receptor (CI-MPR)

[0154] To determine the minimal M6P glycan structures that can still provide high-affinity for CI-MPR, SPR binding studies were performed with immobilized CI-MPR (FIG. **8** and Table 1). The results revealed that the glycopeptide 62 derived from tetrasaccharide oxazoline 8 with a Man6P-α1,

2-Man disaccharide moiety at α1,3-arm of the core β-mannose residue showed strong binding affinity (70 nM) with CI-MPR, which was comparable to that of glycopeptide 66 with a bis-phosphorylated $Man_6GlcNAc_2$ ligand (54 nM). The glycopeptide 65 obtained from the pentasaccharide oxazoline 10 showed similar affinity (82 nM), suggesting that the presence of an additional α1,6-linked mannose moiety did not affect the affinity. Interestingly, when the M6P moiety was α1,3- or α1,6-linked to the next mannosyl residue in the N-glycan, the resulting glycopeptides (60, 61 and 63) showed dramatically decreased affinity for CI-MPR. Taken together, the experimental data suggest that tetrasaccharide oxazoline 8 with a Man6P-α1,2-Man disaccharide moiety at α1,3-arm of the core β-mannose residue appears to be the minimal truncated N-glycan oxazoline derived from the natural structure to provide a high-affinity ligand for CI-MPR. The results further suggest that a Man6P-α1,2-Man disaccharide moiety constitutes an essential structural motif that retains strong binding affinity for the receptor.[39, 40]

TABLE 1

SPR analysis of the binding of M6P-containing glycopeptides with the immobilized CI-MPR receptor. [a]

Compounds	59	60	61	62	63	64	65	66
K_D (μM)	>50 [b]	1.8 [c]	>50 [b]	0.070 ± 0.002	>50 [b]	0.84 [c]	0.082 ± 0.014	0.054 ± 0.006

[a] Serial 2-fold dilution of concentrations (7.8-4000 nM) were performed for the SPR analysis.

[b] No obvious binding was detected up to 50 μM.

[c] Estimated by steady state fitting because the kinetic fitting did not give reliable data. The standard deviations were obtained from three independent experiments.

Example 4: Glycan Remodeling of RNase B and the Affinity of the Glycan Remodeled Protein for CI-MPR

[0155] With the identification of the minimal high-affinity ligand (8), the suitability of this M6P glycan oxazoline was next investigated for glycan remodeling of glycoproteins, first using bovine RNase B as a model substrate, which carries a high-mannose type N-glycan at a single N-glycosylation site (Asn-34) (Scheme 6). RNase B was deglycosylated with wild-type Endo-A to give the homogeneous GlcNAc-RNase B (67). Then the enzymatic reaction between oxazoline 8 and GlcNAc-RNase B under the catalysis of the same enzyme smoothly afforded the phosphorylated glycoprotein 68 in 71% yield, the identity of which was confirmed by ESI-MS (calculated, M=14655; found, M=14656, deconvolution data, FIG. **7**). Although wild-type Endo-A was quite active for hydrolyzing natural Man5-Man9 N-glycan structures, it was found that the M6P glycan oxazoline (8) was only very slowly hydrolyzed by this enzyme, and the resulting glycoprotein was resistant to hydrolysis under the reaction conditions, probably due to the truncation and phosphorylation of the N-glycan. The huge difference in the hydrolytic activities of Endo-A toward the starting glycoprotein and the resulting product, together with its excellent transglycosylation activity toward the truncated M6P-glycan oxazoline, prompted us to devise a one-pot strategy for enzymatic glycan remodeling. Thus, RNase B was incubated with wild-type Endo-A at 30 °C for 30 min before the addition of oxazoline 8 and the reaction mixture was incubated until the transglycosylation was complete. It was found that the deglycosylation of RNase B proceeded quickly, followed by the formation of the desired phosphorylated glycoprotein 68 as a result of transglycosylation. The one-pot protocol simplified the procedure by omitting the purification of the intermediate after deglycosylation, thus improving the overall efficiency of the glycan remodeling approach. SPR binding experiments revealed that the M6P-containing RNase B (68) showed strong affinity for CI-MPR (K_D=15.8 nM, FIG. **9**), which, again, was comparable to that of the glycoprotein with a large bis-M6P-$Man_6GlcNAc_2$ glycan.[30] The results suggest that a simple M6P tetrasaccharide oxazoline is sufficient to enable the glycan remodeling of glycoprotein with high-affinity binding to CI-MPR in a one-pot manner. See FIG. **17**, scheme 6.

Example 5: One-Pot and Glycan-Selective Remodeling of the Recombinant Human Acid α-Glucosidase (rhGAA, Lumizyme) Using Wild-Type Endo-A and Endo-F3

[0156] Following the promising model study with RNase B, further studies were performed to evaluate the feasibility of the one-pot enzymatic M6P-glycan remodeling on the recombinant human acid α-glucosidase (rhGAA; Lumizyme, Sanofi Genzyme), a therapeutic, multiply glycosylated lysosomal enzyme used for the treatment of Pompe disease.[22] rhGAA produced in CHO cells has seven N-glycosylation sites, 31 of which two sites (N233 and N470) reportedly contain high-mannose type glycans, while the rest are mainly occupied by core-fucosylated complex type N-glycans.[19] Lumizyme, which is currently used for the enzyme replacement therapy of Pompe disease, contains relatively low amounts of high-mannose M6P-glycans, thus limiting its targeting and the overall therapeutic efficacy. The low M6P contents partially explain why up to 20-fold higher dose is usually required for the treatment of Pompe disease than those of the lysosomal enzymes used for the treatment of other LSDs.[22] Considering the substrate specificity of different endoglycosidases, studies were designed to selectively modify the high-mannose type N-glycans but leave the complex type N-glycans unchanged, or keep the high-mannose type N-glycans while acting on the complex type N-glycans selectively. Based on the success in modification of RNase B, wild-type Endo-A offers an excellent choice to selectively trim the high-mannose type glycans and install simultaneously the synthetic M6P-glycan via a one-pot strategy in view of its substrate specificity.[41] Thus, the commercial rhGAA was treated with Endo-A, followed by addition of several portions of oxazoline 8 at 30° C. in one pot. The resulting reaction mixture was treated with Glutathione Agarose to remove the GST-tagged Endo-A, and the cleaved glycans and salts were removed by ultrafiltration (Scheme 7). Glycan analysis revealed the removal of high-

mannose type N-glycans and introduction of the M6P-glycan after transglycosylation without affecting the complex type N-glycans (FIG. **2**A, FIG. **2**C, and FIG. **10**). This result confirmed the efficiency of the site-selective glycan remodeling of a multiply glycosylated therapeutic protein.

[0157] Since the major glycoforms of rhGAA are core-fucosylated complex type N-glycans, studies were performed to selectively remodel the core-fucosylated complex type N-glycans with M6P-glycan and to keep the original phosphorylated high-mannose type N-glycans unchanged. For this purpose, these studies employed Endo-F3, an endoglycosidase from *Elizabethkingia meningoseptica* that efficiently hydrolyzes core-fucosylated complex type N-glycans but is unable to cleave high-mannose type N-glycans.[42-45] As an initial experiment, Endo-F3 indeed efficiently transferred the minimal M6P-tetrasaccharide oxazoline (8) to a model core-fucosylated GlcNAc-peptide (Fuca1, 6GlcNAc-CD52) and, interestingly, the resulting M6P-glycopeptide was resistant to hydrolysis by this enzyme (FIG. **11**). This preliminary study prompted us to test a one-pot strategy for selective glycan remodeling of rhGAA with Endo-F3. Thus, the commercial rhGAA was treated with Endo-F3 to selectively remove the core-fucosylated N-glycans, presumably at the N140, N390, N652, and N925 sites based on previous site-specific glycosylation profiling analysis,[16, 19] followed by three portions of tetrasaccharide oxazoline 8 every 2 hours at 30° C. (Scheme 7). The resulting reaction mixture was purified by HisTrap column to remove the His-tagged Endo-F3, and the cleaved glycans and extra salts were removed by ultrafiltration. Notably, Endo-F3-deglycosylated rhGAA was unstable and prone to precipitation. As a result, the one-pot simultaneous deglycosylation/transglycosylation strategy appeared to be much more efficient than a stepwise method that required an isolation of the deglycosylated intermediate. Glycan analysis confirmed the removal of complex type N-glycans and introduction of the M6P-glycan without affecting the high-mannose type N-glycans (FIG. **2**E and FIG. **10**). The Endo-F3-based glycan remodeling method is complementary to the Endo-A-based glycan remodeling in terms of the N-glycan selectivity. Given the fact that most of the therapeutic enzymes used in ERTs are multiply glycosylated and usually carry both high-mannose type and complex type N-glycans, the present selective glycan remodeling method provides a general platform for a single-step, site- and glycan-selective M6P-glycan remodeling of most lysosomal enzymes with a more homogeneous product, which appears to be superior to the existing chemical conjugations with natural and synthetic M6P ligands.[16, 24] See FIG. **18**, scheme 7.

Example 6: CI-MPR Binding of the M6P Glycan-Remodeled rhGAA

[0158] SPR experiments indicated that the native rhGAA had a notable affinity for CI-MPR (K_D=14.0 nM) (FIG. **2**B and FIG. **12**). Upon treatment with wild-type Endo-A to remove the high-mannose N-glycans including the M6P glycans, the deglycosylated rhGAA lost its binding affinity (FIG. **12**). However, after Endo-A catalyzed transglycosylation with the M6P-tetrasaccharide oxazoline (8), the remodeled rhGAA (69) exhibited a 6-fold enhanced affinity for the receptor (K_D=2.3 nM, FIG. **2**D). On the other hand, the Endo-F3-remodeled rhGAA (70) showed a 20-fold enhanced affinity (K_D=0.63 nM, FIG. **2**F). These results suggested that introduction of additional M6P-glycans into rhGAA resulted in further enhancement of affinity for the receptor. Previous studies have also shown that a novel rhGAA (designated as ATB200) carrying a higher M6P content that binds the CI-MPR with high affinity (apparent K_D, ~2-4 nM), exhibited enhanced cellular uptake and led to much improved glycogen reduction and reversal of muscle pathology in preclinical models.[46] As a result, it was expected that the Endo-A remodeled rhGAA might exhibit comparable in vivo potency as ATB200, while the Endo-F3 remodeled enzyme might demonstrate much better therapeutic efficacy than ATB200 and commercial rhGAA, due to its much higher affinity for CI-MPR.

Example 7: Enzyme Activity of the M6P Glycan-Remodeled rhGAA

[0159] To confirm if the remodeled enzymes still maintained their catalytic activity after M6P-glycan remodeling, the α-glucosidase activity of the commercial rhGAA, the Endo-A remodeled rhGAA (69), and the Endo-F3 remodeled rhGAA (70) were assessed using 4-methylumbelliferyl-α-D-glucopyranoside (4-MUG) as the substrate.[24] The results indicated that the M6P glycan remodeled rhGAA maintained full enzyme activity as the parent rhGAA (FIG. **3**). These data confirmed that the glycoengineering process was mild enough without denaturing the enzyme, and the resulting M6P-glycan remodeled enzyme was stable.

Example 8: Evaluation of the Biological Effect of the Endo-A and Endo-F3 Remodeled rhGAA in an In Vitro Model of Pompe Disease

[0160] Skeletal muscle is a major tissue affected in all forms of Pompe disease, and its response to the currently available therapy with the recombinant human GAA (rhGAA; Lumizyme) is not satisfactory.[47] To evaluate the effect of the Endo-A (69) and Endo-F3 (70) remodeled rhGAA in the disease-relevant muscle cells, GAA-deficient multinucleated myotubes (KO) were used as an in vitro cell model system for Pompe disease.[48, 49] These myotubes are formed from conditionally immortalized myoblasts derived from the GAA knockout mice; unlike myoblasts, the differentiated myotubes replicate the primary defect of the disease, namely, the enlargement of glycogen-laden lysosomes. This physiologically relevant in vitro cell model system has been shown to closely replicate the pathogenic mechanisms of muscle tissue abnormalities in Pompe disease.[48, 49]

[0161] Muscle cells were exposed to the commercial rhGAA (Lumizyme), the Endo-A remodeled rhGAA (69), and Endo-F3 remodeled rhGAA (70) (5 μM for 24 hours), which reach lysosomes via mannose 6-phosphate-mediated

endocytosis. KO myotubes treated with Lumizyme and the glycoengineered proteins (69 and 70) were lysed, and the GAA activity was quantified using 4-Methylumbelliferyl-α-D-glucopyranoside, a fluorogenic substrate that is routinely used for the GAA assay in the diagnosis of Pompe disease. GAA activity in the cell lysates increased significantly following incubation with both M6P-glycan remodeled proteins (69 and 70), whereas only a slight increase (statistically insignificant) was observed in Lumizyme-treated cells compared to the background level in the untreated cells (FIG. **4**A). These results are consistent with the enhanced affinity of the M6P-glycan remodeled enzymes (69 and 70) to the CI-MPR receptor (FIGS. **2**A-**2**F). The effect of the Endo-F3 remodeled rhGAA (70) was more pronounced compared to that of Endo-A remodeled protein (69), suggesting its better cellular uptake (FIG. **4**A). Western blot with anti-human GAA antibodies revealed the presence of the processed, mature lysosomal form of rhGAA (76 kDa) in cells treated with the Endo-F3 remodeled protein (70) (FIG. **4**B, left panel); a weaker band was also detected in cell lysates following incubation with the Endo-A remodeled enzyme (69). In contrast, the 76 kDa form was barely detectable in cells treated with Lumizyme. The available anti-human GAA antibodies detected a strong non-specific band in mouse muscle cells as indicated by its presence in both WT and GAA-deficient cells (FIG. **4**B, right panel). Previous studies have shown that the mature 76 kDa form of GAA has a higher affinity and activity toward glycogen.[31, 50]

[0162] The immunoblot also showed that the molecular weight of the internalized GAA precursor appeared to be lower than the expected 110 kDa in the samples treated with the Endo-F3 remodeled rhGAA (70) compared to those treated with Lumizyme or the Endo-A remodeled rhGAA (69). The lower molecular weight of the Endo-F3 remodeled enzyme (70) was confirmed by Western analysis of the three recombinant proteins stained with anti-human GAA antibodies (FIG. **4**C). These data were also consistent with the MALDI-TOF MS analysis of the glycan-remodeled rhGAA (69 and 70), which showed that the Endo-A remodeled rhGAA (69) had an average molecular mass of ca. 110 kDa, while the Endo-F3 remodeled rhGAA (70) had a molecular mass of ca. 108 kDa (FIG. **13**). This observation was expected, as Endo-F3 based remodeling replaced most of the complex type N-glycans in the rhGAA with a much smaller (pentasaccharide) M6P N-glycan while the Endo-A based remodeling only changed the two high-mannose N-glycans to the M6P N-glycans. The efficient lysosomal trafficking of the remodeled proteins was associated with a significant reduction in the levels of both endosomal (Rab5) and lysosomal (LAMP1) markers, suggesting a reversal of lysosomal swelling; in contrast, the levels of these markers in Lumizyme-treated cells remained the same as in the untreated GAA-deficient cells (FIG. **4**D).

[0163] The effect of the Endo-A and Endo-F3 remodeled rhGAA was confirmed by immunostaining of myotubes with Lamp1. Enlarged lysosomes were seen in cells treated with Lumizyme but not in those treated with the remodeled enzymes (69 and 70) (FIG. **5**A; see also additional images in FIG. **14**). Finally, the degree of glycogen reduction achieved with Endo-A and Endo-F3 remodeled rhGAA (69 and 70) was significantly greater compared to that with the commercial rhGAA. Importantly, glycogen content in the diseased cells returned to the WT level following treatment with Endo-F3 remodeled rhGAA (70) (FIG. **5**B). Taken together, these in vitro data indicated that upon efficient internalization, the M6P-glycan remodeled proteins, in particular, the Endo-F3 remodeled rhGAA, could efficiently traffic to their correct cellular destination, the lysosome, and do their job there, i.e., break down the accumulated glycogen. The therapeutic potential of the M6P glycan-remodeled rhGAA in lysosomal glycogen reduction and reversal of muscle pathology will be further evaluated in Pompe disease animal models in future studies.

[0164] In conclusion, described herein are the chemical synthesis of an array of mannose-6-phosphate (M6P) containing N-glycan oxazolines and their use as donor substrates for chemoenzymatic synthesis of M6P-containing glycopeptides and for the glycan remodeling of a therapeutic lysosomal enzyme (rhGAA). The present study revealed a Man6P-α1,2-Man disaccharide as an essential structural motif for high-affinity binding to the M6P receptor (CI-MPR). Structure-activity relationship studies identified a tetrasaccharide oxazoline carrying this M6P disaccharide motif as the minimal donor substrate for efficient transglycosylation to give high-affinity M6P ligands. The discovery on the resistance of the M6P product to the hydrolysis by wild-type Endo-A and Endo-F3, coupled with the excellent hydrolysis activity of the wild-type enzymes on high-mannose and core-fucosylated complex type N-glycans, respectively, enabled a site-selective and one-pot conjugation of the high-affinity M6P glycan ligands either at the high-mannose or complex type N-glycosylation sites in the multiply glycosylated protein, giving structurally well-defined product. The Endo-A and Endo-F3 remodeled rhGAAs maintained full enzyme activities and demonstrated 6- and 20-fold enhanced binding affinities for CI-MPR, respectively. Moreover, by using an in vitro cell model system for Pompe disease, it was demonstrated that the M6P-glycan remodeled rhGAA showed significantly enhanced cellular uptake over the commercial Lumizyme and exhibited much more efficient glycogen reduction in lysosomes than Lumizyme. While the therapeutic potential of the M6P glycan-remodeled rhGAA may be further evaluated in Pompe disease animal models, the present study provides a general and efficient method for site-selective M6P-glycan remodeling of recombinant lysosomal enzymes to achieve enhanced M6P receptor binding and cellular uptake, which holds a great promise for improved overall therapeutic efficacy of enzyme replacement therapy.

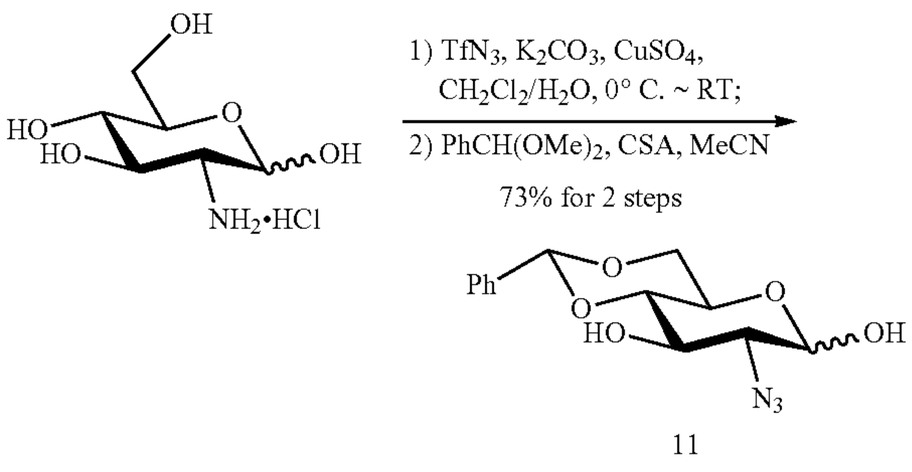
Example 9: Chemical Synthesis

Materials and Methods

[0165] All chemicals, reagents, and solvents were purchased from Sigma-Aldrich and TCI and unless specially noted applied in the reaction without further purification. TLC was performed using silica gel on glass plates (Sigma-Aldrich), and spots were detected under UV light (254 nm) then charring with 5% (v/v) sulfuric acid in EtOH or cerium molybdate stain (CAM) followed by heating at 150° C. Silica gel (200-425 mesh) for flash chromatography was purchased from Sigma-Aldrich. NMR spectra were recorded on a 400 MHz spectrometer (Bruker, Tokyo, Japan) with $CDCl_3$ or D_2O as the solvent. The chemical shifts were assigned in ppm, and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are reported in Hertz. MALDI-TOF was performed on a Bruker Autoflex Speed Mass Spectrometer in positive reflectron mode with DHB (ACN/H_2O=1:1) as the matrix. HRMS was performed on an Exactive Plus Orbitrap Mass Spectrometer (Thermo Scientific) equipped with a C18 column. Analytical RP-HPLC was performed on a Waters 626 HPLC instrument with a C18 column (3.5 μm, 4.6×250 mm) at 50° C. The column was eluted with a linear gradient containing 0.1% FA for 30 min at the flow rate of 1.0 mL/min. Preparative HPLC was performed with a Waters 600 HPLC instrument and Waters C18 columns (5.0 μm, 10×250 mm; 7.0 μm, 19×300 mm). The column was eluted with a suitable gradient of MeCN—H_2O containing 0.1% TFA or FA at a flow rate of 4 mL/min or 10 mL/min. DIONEX HPAEC-PAD was performed on a Thermo Scientific Dionex ICS-6000 instrument and a PA200 column using a gradient of A (100 mM NaOH) and B (100 mM NaOH and 250 mM NaOAc) at a flow rate of 0.5 mL/min (0-60% B, 30 min).

2-Azido-4,6-O-benzylidene-2-deoxy-αβ-D-glucopyranoside (11)

[0166]



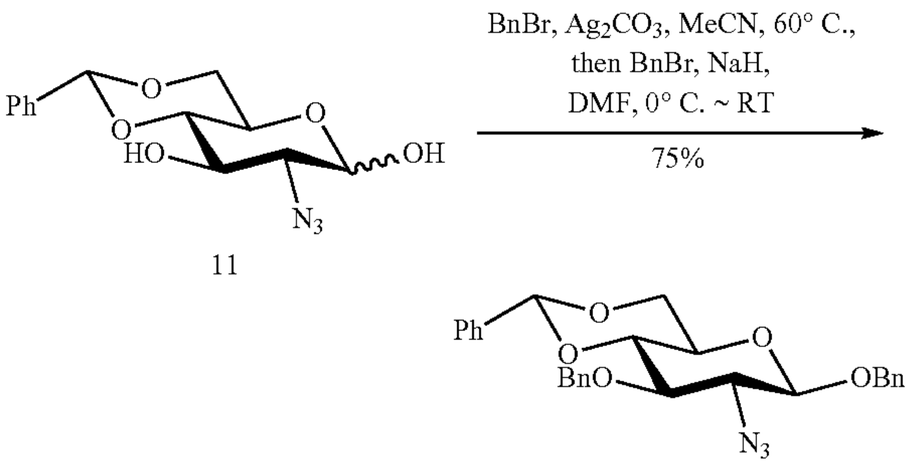
11

[0167] In situ preparation of TfN_3: To a vigorously stirring solution of NaN_3 (3.00 g, 46.1 mmol) in H_2O/CH_2Cl_2 (1:1, 10 mL) was dropwise added Tf_2O (1.50 mL, 8.95 mmol) at 0° C. The resulting mixture turned into white cloudy solution and was stirred at 0° C. for a further 1.5 h. The organic layer was washed with water and saturated Na_2CO_3 (aq.), and the resulting solution of TfN_3 (in about 10 mL of CH_2Cl_2) was directly used in the next step without further purification.

[0168] Cu^{II}-catalyzed diazo transfer and protection with benzylidene: The freshly prepared solution of TfN_3 (in CH_2Cl_2) was added to a solution of D-glucosamine hydrochloride (1.00 g, 4.65 mmol), K_2CO_3 (0.75 g, 5.43 mmol), and a catalytic amount of $CuSO_4$ (10 mg) in water (5 mL) at 0° C. Then, the ice bath was removed and MeOH was added to make the reaction homogeneous. After vigorous stirring at RT for 18 h, the mixture was passed through a pad of Celite and the filtrate was concentrated under reduced pressure to give a dry residue that was purified by a short column of silica gel (CH_2Cl_2/MeOH=5:1~4:1) to give the crude azide product. To the crude azide product in MeCN (10 mL) was added (+)−10-Camphorsulfonic acid (195 mg, 0.84 mmol) and benzaldehyde dimethyl acetal (2.0 mL, 13.3 mmol), and the mixture was stirred at room temperature overnight. After the completion of the reaction as monitored by TLC, triethylamine was added to quench the reaction. Flash chromatography (hexanes/EtOAc=2:1) afforded the product 11 as white solid (1.00 g, 73% for 2 steps, mixture of a/ß isomers, 1.7:1). R_f=0.20 (hexanes/EtOAc=2:1). Spectroscopic data were in agreement with literature values.[1]

Benzyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (12)

[0169]



11

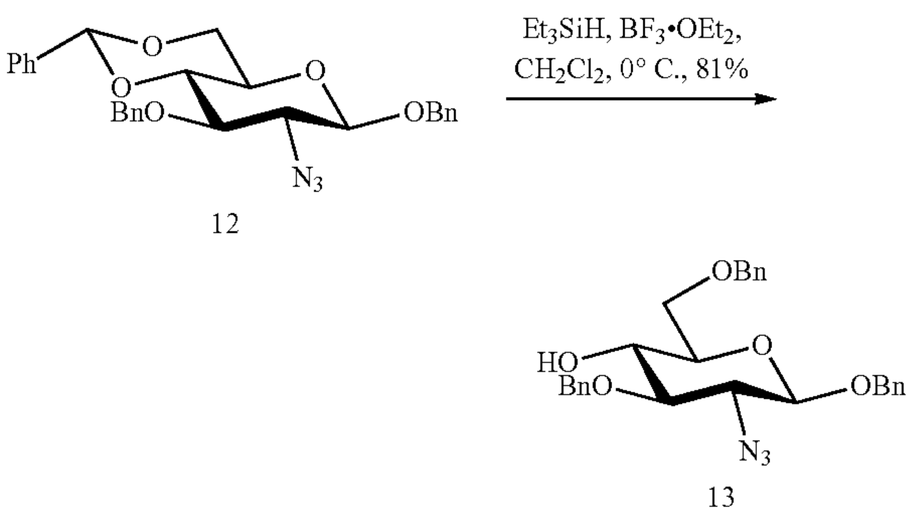
12

[0170] To a solution of compound 11 (952 mg, 3.25 mmol) in anhydrous acetonitrile (20 mL) was added Ag_2CO_3 (4.48 g, 16.24 mmol) and benzyl bromide (1.54 mL, 13.00 mmol), the mixture was kept in dark and heated to 60° C. overnight. When TLC showed the disappearance of the starting material, indicating the complete protection of the anomeric hydroxyl, the mixture was filtered through a pad of Celite and the filtrate was concentrated to dryness. The residue was dissolved in dry N,N-dimethylformamide (20 mL) and cooled to 0° C., sodium hydride (325 mg, 8.13 mmol) and benzyl bromide (776 μL, 6.50 mmol) were added successively, and the mixture was slowly warmed to room temperature. After the completion of the reaction as monitored

by TLC, MeOH (0.5 mL) was added to quench the excess sodium hydride. The reaction was diluted with CH_2Cl_2, successively washed with H_2O and brine and dried over anhydrous Na_2SO_4. Flash column chromatography (hexanes/EtOAc=10:1~8:1) gave 12 (1.151 g, 75%) as white solid. R_f=0.30 (hexanes/EtOAc=10:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.54-7.52, 7.43-7.30 (15H, m, Ar—H), 5.63 (1H, s, PhCH), 4.97 (1H, d, $PhCH_2$, J=11.8 Hz), 4.96 (1H, d, $PhCH_2$, J=11.2 Hz), 4.84 (1H, d, $PhCH_2$, J=11.2 Hz), 4.73 (1H, d, $PhCH_2$, J=11.8 Hz), 4.48 (1H, d, H−1, J=7.8 Hz), 4.42 (1H, dd, J=10.5 Hz, J=5.0 Hz), 3.86 (1H, dd, J=10.3 Hz, J=10.3 Hz), 3.77 (1H, dd, J=9.2 Hz, J=9.2 Hz), 3.61-3.56 (2H, m), 3.43 (1H, m); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 137.87, 137.17, 136.51, 129.10, 128.57, 128.41, 128.33, 128.23, 128.17, 128.07, 127.91, 126.04, 101.37, 101.14, 81.61, 79.06, 74.96, 71.43, 68.63, 66.25; MALDI-TOF: $[M+Na]^+$ calcd for $C_{27}H_{27}N_3NaO_5^+$, 496.18; found, 495.91.

Benzyl 2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (13)

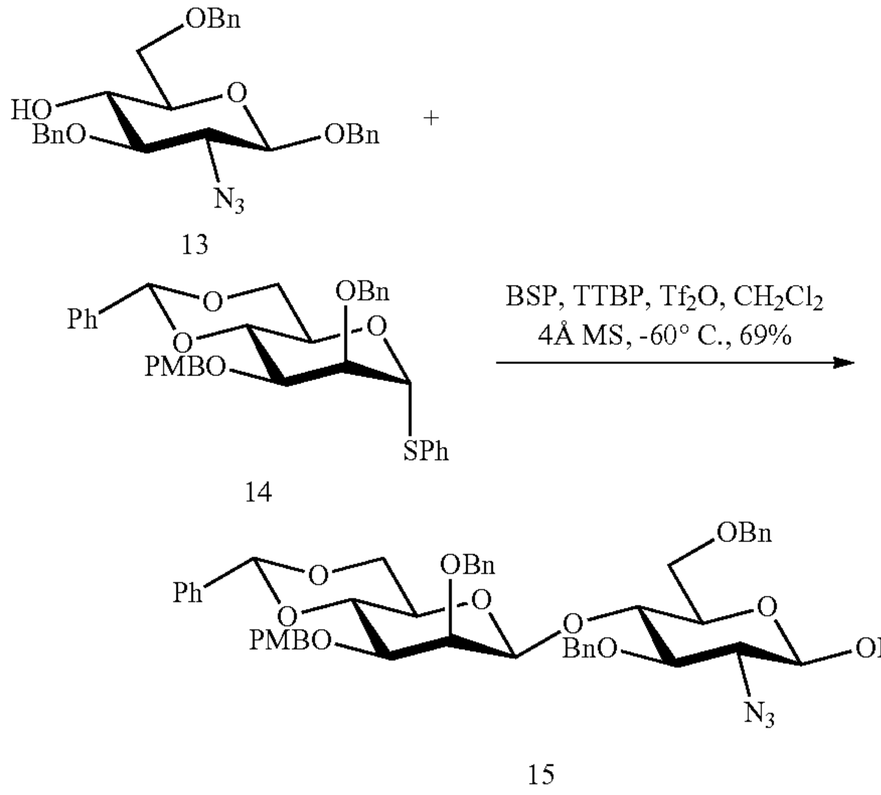
[0171]



[0172] To a solution of compound 12 (500 mg, 1.056 mmol) in anhydrous CH_2Cl_2 (12 mL) was added triethylsilane (1.01 mL, 6.34 mmol) and $BF_3{\cdot}OEt_2$ (0.67 mL, 5.28 mmol) at 0° C., the mixture was stirred at this temperature for 3 h and quenched by triethylamine. The residue was concentrated and purified by flash column chromatography (hexanes/EtOAc=10:1~4:1) to give 13 (406 mg, 81%) as colorless syrup. R_f=0.30 (hexanes/EtOAc=4:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.44-7.35 (15H, m, Ar—H), 4.98-4.93 (2H, m, $PhCH_2$), 4.81 (1H, d, $PhCH_2$, J=11.3 Hz), 4.72 (1H, d, $PhCH_2$, J=11.9 Hz), 4.68-4.59 (2H, m, $PhCH_2$), 4.40 (1H, d, H−1, J=8.1 Hz), 3.79 (2H, m), 3.69 (1H, m), 3.52-3.42 (2H, m), 3.28 (1H, dd, J=9.3 Hz, J=9.3 Hz), 2.70 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.13, 137.76, 136.77, 128.64, 128.53, 128.51, 128.15, 128.07, 128.05, 128.02, 127.90, 127.77, 100.64, 82.62, 75.13, 74.06, 73.77, 71.96, 71.02, 70.17, 65.79; MALDI-TOF: $[M+Na]^+$ calcd for $C_{27}H_{29}N_3NaO_5$, 498.20; found, 498.18.

Benzyl 2-O-benzyl-4,6-O-benzylidene-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (15)

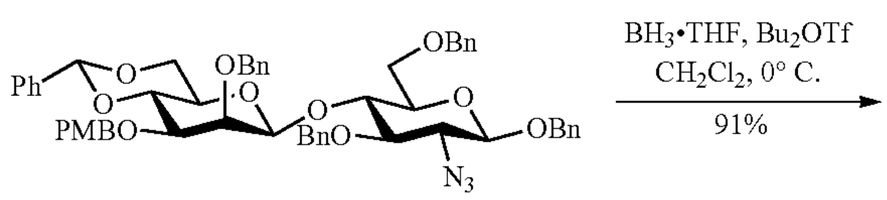
[0173]



[0174] To a solution of compound 14[2] (581 mg, 1.019 mmol) in anhydrous CH_2Cl_2 (18 mL) was added activated 4 Å molecular sieves (2.0 g) under argon atmosphere, the mixture was stirred for 2 h at room temperature, then cooled to −60° C. BSP (236 mg, 1.13 mmol) and TTBP (427 mg, 2.05 mmol) were added, and the solution was kept at −60° C. for 40 min before Tf_2O (206 μL, 1.23 mmol) was added. After 20 min, a solution of compound 13 (323 mg, 0.68 mmol) in CH_2Cl_2 (3.0 mL) was added and the mixture was stirred at −60° C. for 3 h. After the completion of the reaction as monitored by TLC, the mixture was filtered through a Celite pad. The filtrate was poured into saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (hexanes/EtOAc=10:1~5:1) to afford 15 (441 mg, 69%) as a colorless syrup. R_f=0.40 (hexanes/EtOAc=4:1); Spectroscopic data were in agreement with literature values. (31 MALDI-TOF: $[M+Na]^+$ calcd for $C_{55}H_{57}N_3NaO_{11}^+$, 958.39; found, 958.53.

Benzyl 2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (16)

[0175]



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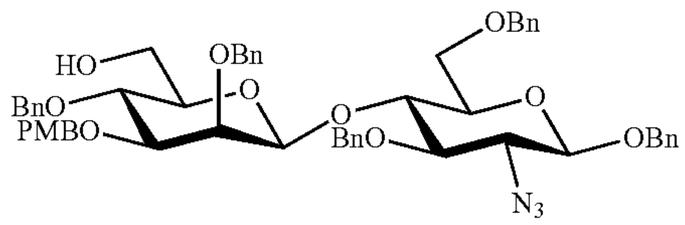
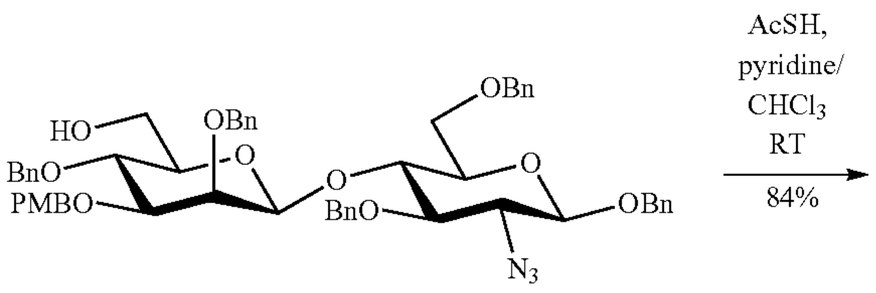


16

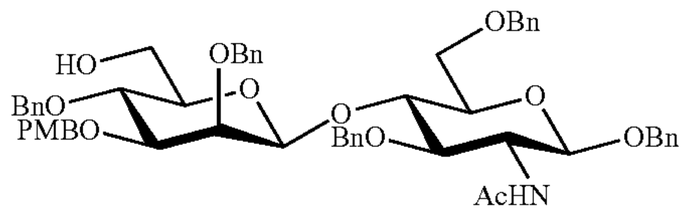
[0176] To a solution of compound 15 (300 mg, 0.321 mmol) in BH_3·THF (4.0 mL) was added a solution of Bu_2BOTf in CH_2Cl_2 (1 M, 642 μL) under argon atmosphere at 0° C. and the mixture was stirred at 0° C. for 40 min when TLC indicated the completion of the reaction. Et_3N (300 μL) was added to the reaction followed by careful addition of MeOH (600 μL). The mixture was co-evaporated with MeOH three times and the residue was purified by flash chromatography (hexanes/EtOAc=5:1~2:1) to afford 16 (274 mg, 91%) as a colorless syrup. R_f=0.30 (hexanes/EtOAc=3:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.50-7.28 (27H, m, Ar—H), 6.93 (2H, m, Ar—H), 5.16 (1H, d, $PhCH_2$, J=10.8 Hz), 5.01 (1H, d, $PhCH_2$, J=12.1 Hz), 4.97-4.86 (3H, m, $PhCH_2$), 4.78 (1H, d, $PhCH_2$, J=12.1 Hz), 4.75-4.64 (3H, m, $PhCH_2$), 4.55-4.52 (3H, m, $PhCH_2$), 4.40 (1H, d, J=8.1 Hz), 4.01 (1H, dd, J=9.3 Hz, J=9.3 Hz), 3.89-3.75 (6H, m), 3.73-3.67 (2H, m), 3.57 (1H, dd, J=8.2 Hz, J=8.2 Hz), 3.45-3.39 (4H, m), 3.22-3.18 (1H, m), 1.97 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.31, 138.76, 138.52, 138.47, 137.75, 136.93, 130.38, 129.20, 128.64, 128.56, 128.45, 128.38, 128.26, 128.17, 128.06, 128.03, 127.91, 127.78, 127.69, 127.65, 127.51, 113.89, 100.76, 100.51, 82.34, 81.50, 77.08, 75.80, 75.31, 75.10, 74.90, 74.83, 74.57, 73.71, 71.65, 70.93, 68.54, 65.96, 62.22, 55.34; MALDI-TOF: $[M+Na]^+$ calcd for $C_{55}H_{59}N_3NaO_{11}^+$, 960.40; found, 959.98.

Benzyl 2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (17)

[0177]



16

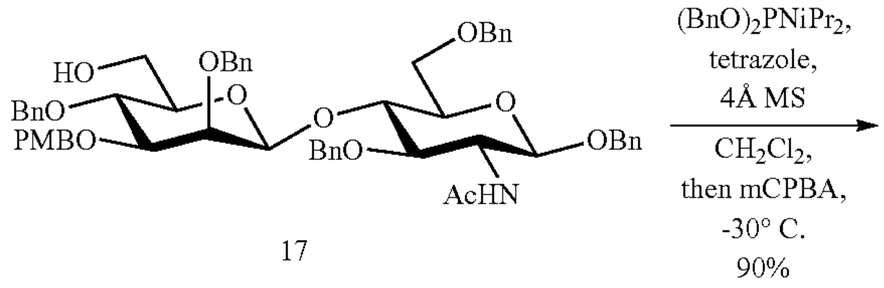


17

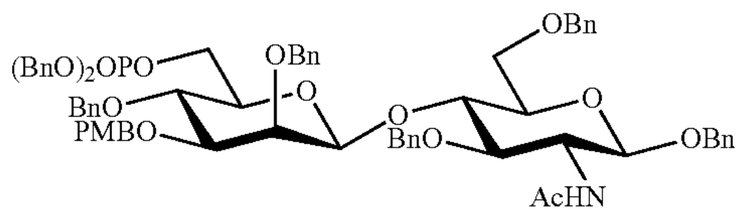
[0178] A solution of compound 16 (133.5 mg, 0.142 mmol) in a mixture of AcSH/pyridine/$CHCl_3$ (0.8 mL/0.6 mL/0.8 mL) was stirred at room temperature for 18 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=4:1~3:2) to afford compound 17 (113.8 mg, 84%) as colorless syrup. R_f=0.30 (hexanes/EtOAc=2:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.44-7.43, 7.37-7.23 (27H, m, Ar—H), 6.88-6.86 (2H, m, Ar—H), 5.75 (1H, d, NH, J=8.0 Hz), 4.98-4.81 (6H, m, $PhCH_2$), 4.65-4.59 (4H, m, $PhCH_2$), 4.53-4.47 (4H, m, $PhCH_2$), 4.16 (1H, dd, J=7.8 Hz, J=7.8 Hz), 3.90 (1H, dd, J=7.0 Hz, J=7.0 Hz), 3.86-3.78 (6H, m), 3.73-3.69 (3H, m), 3.64-3.58 (1H, m), 3.53-3.48 (1H, m), 3.42-3.39 (1H, m), 3.22-3.18 (1H, m), 2.09 (1H, s), 1.76 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.54, 159.25, 138.78, 138.63, 138.37, 137.91, 137.60, 130.28, 129.17, 128.51, 128.39, 128.33, 128.26, 128.15, 128.02, 127.88, 127.74, 127.66, 127.61, 127.54, 113.84, 101.08, 99.14, 82.18, 77.78, 75.71, 75.47, 75.13, 75.08, 74.76, 74.62, 73.75, 73.57, 71.59, 70.71, 69.38, 62.28, 55.29, 23.38; MALDI-TOF: $[M+Na]^+$ calcd for $C_{57}H_{63}NNaO_{12}^+$, 976.42; found, 976.00.

Benzyl 2,4-di-O-benzyl-6-O-dibenzylphosphonato-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (18)

[0179]



17



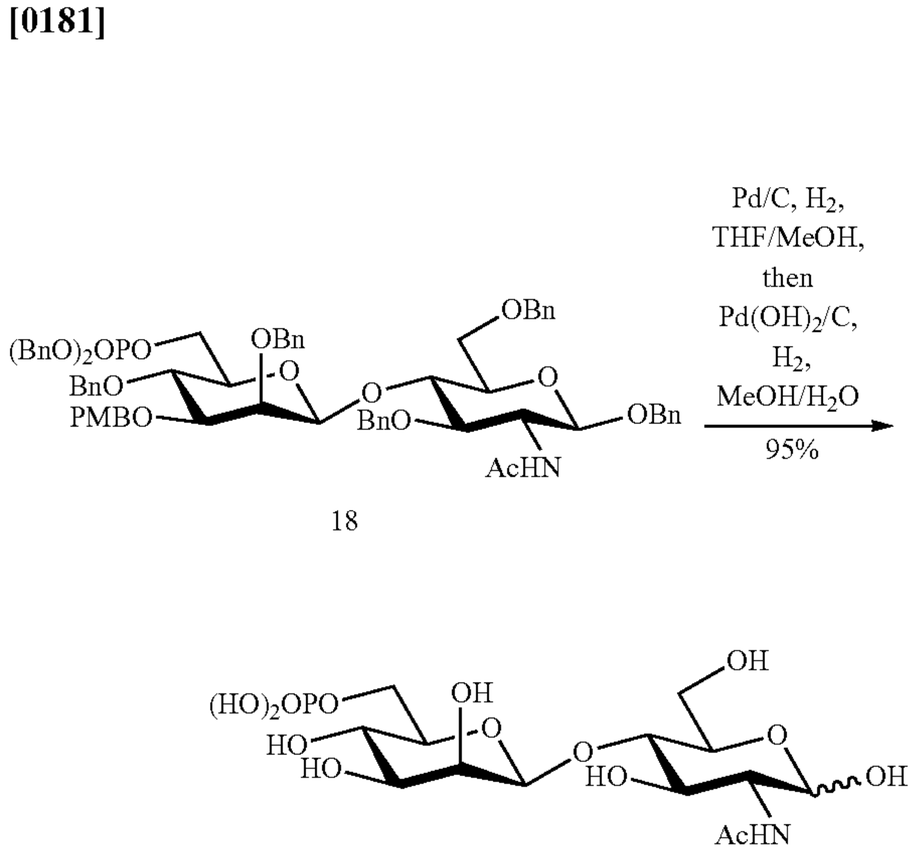
18

[0180] To a solution of compound 17 (50.0 mg, 0.052 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added activated 4 Å molecular sieves (200 mg) and tetrazole (0.45 M in MeCN, 582 μL) and the mixture was stirred at room temperature for 1.5 h before $(BnO)_2PNiPr_2$ (70.6 μL) was added. The resulting mixture was further stirred overnight under argon atmosphere at room temperature until the complete disappearance of the starting material. Then the reaction was cooled to −30° C., and mCPBA (77 wt %, 61.5 mg)

was added, the reaction mixture was stirred at this temperature for 1 h and then filtered through a Celite pad. The filtrate was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (aq.), dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography to give compound 18 (56.8 mg, 90%) as colorless syrup. R_f=0.30 (hexanes/Acetone=2:1); ^1H NMR (400 MHZ, $CDCl_3$) δ 7.42-7.40, 7.34-7.20 (37H, m, Ar—H), 6.87-6.85 (2H, m, Ar—H), 5.96 (1H, d, NH, J=8.3 Hz), 4.99-4.92 (4H, m, $PhCH_2$), 4.89 (1H, d, J=11.9 Hz, $PhCH_2$), 4.88 (1H, d, J=10.8 Hz, $PhCH_2$), 4.85-4.82 (3H, m, $PhCH_2$), 4.79 (1H, d, J=11.8 Hz, $PhCH_2$), 4.65 (1H, d, J=11.8 Hz, $PhCH_2$), 4.60-4.55 (3H, m), 4.52 (1H, d, J=11.7 Hz, $PhCH_2$), 4.47-4.41 (2H, m), 4.25-4.20 (1H, m), 4.19-4.12 (1H, m), 4.06 (1H, dd, J=7.1 Hz, J=7.1 Hz), 3.97 (1H, dd, J=6.9 Hz, J=6.9 Hz), 3.85-3.79 (6H, m), 3.77-3.71 (2H, m), 3.40 (1H, dd, J=2.8 Hz, J=9.3 Hz), 3.35-3.32 (1H, m), 1.69 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.07, 159.30, 138.87, 138.51, 138.17, 138.08, 137.80, 135.85, 135.78, 130.05, 129.24, 128.44, 128.39, 128.29, 128.24, 128.21, 128.11, 128.05, 128.03, 127.95, 127.93, 127.77, 127.73, 127.69, 127.58, 127.54, 127.38, 113.85, 100.92, 99.61, 81.98, 77.19, 77.13, 75.19, 75.11, 74.93, 74.54, 74.38, 74.30, 73.86, 73.49, 72.73, 71.49, 70.45, 69.68, 69.34, 69.29, 66.68, 66.63, 55.28, 53.58, 23.17; 31P NMR (146 MHz, $CDCl_3$) δ −1.20; MALDI-TOF: $[M+Na]^+$ calcd for $C_{71}H_{76}NNaO_{15}P^+$, 1236.48; found, 1236.25.

6-O-phosphonato-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-αβ-D-glucopyranoside (19)

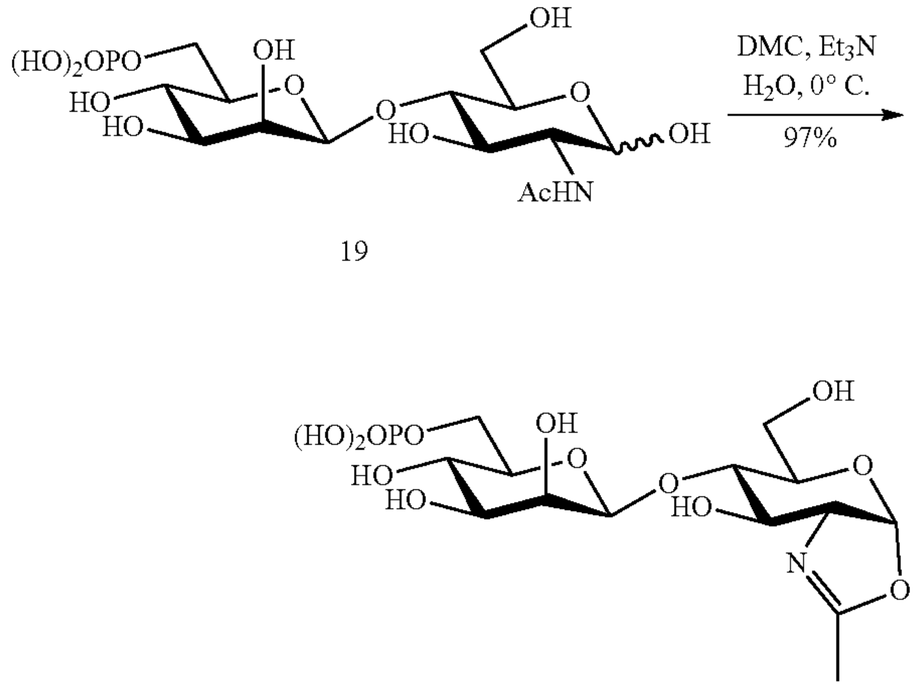
[0181]



[0182] A mixture of compound 18 (56.8 mg, 0.047 mmol) and Pd/C (10 wt. % loading, 30 mg) in MeOH (2.0 mL) and THF (2.0 mL) was stirred under H_2 atmosphere for 21 h. The reaction mixture was filtered through a Celite pad, then concentrated to dryness. The mixture of the residue and $Pd(OH)_2/C$ (20 wt. % loading, 30 mg) in MeOH (2.5 mL) and H_2O (2.5 mL) was stirred under H_2 atmosphere for further 21 h. The reaction mixture was filtered through a Celite pad. The filtrate was concentrated to dryness then dissolved in H_2O and lyophilized. The crude product was purified on a Sephadex G-10 column by elution with H_2O. Fractions containing the product were pooled and lyophilized to give compound 19 (20.5 mg, 95%) as white solid. R_f=0.50 (n-BuOH/EtOH/H_2O/AcOH=1:1:1:0.05); ^1H NMR (400 MHZ, D_2O) δ 5.15 (0.54H, d, J=3.4 Hz), 4.65 (0.44H, d, J=8.2 Hz), 4.05-3.97 (2.15H, m), 3.96-3.92 (1.07H, m), 3.90-3.86 (1.28H, m), 3.83-3.78 (1.12H, m), 3.77-3.74 (0.76H, m), 3.73-3.71 (0.53H, m), 3.71-3.68 (0.77H, m), 3.68-3.63 (2.64H, m), 3.62-3.59 (1.06H, m), 3.54-3.51 (0.44H, m), 3.45-3.41 (0.99H, m), 1.97 (3H, s); ^{13}C NMR (100 MHZ, D_2O) δ 174.36, 174.06, 100.01, 99.86, 94.55, 90.04, 79.36, 78.89, 75.10, 75.05, 74.21, 72.10, 71.76, 70.20, 70.17, 69.63, 68.64, 65.72, 63.06, 59.97, 59.83, 55.80, 53.35, 21.81, 21.50; 31P NMR (146 MHZ, D_2O) δ 2.76 (overlapped signals); HRMS: $[M+H]^+$ calcd for $C_{14}H_{27}NO14P^+$, 464.1164; found, 464.1169.

2-Methyl-[6-O-phosphonato-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline (5)

[0183]

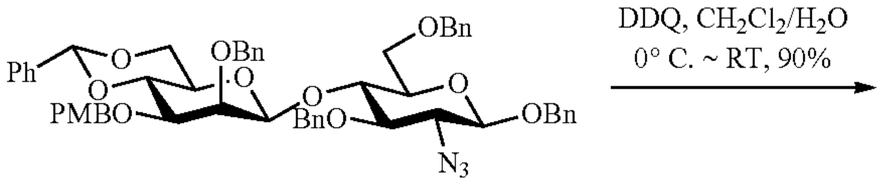


[0184] To a solution of compound 19 (5.0 mg, 0.011 mmol) in H_2O (300 μL) were added Et_3N (60.5 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 36.6 mg) at 0° C. The reaction mixture was monitored by DIONEX HPAEC-PAD. After 2 h, the HPAEC analysis

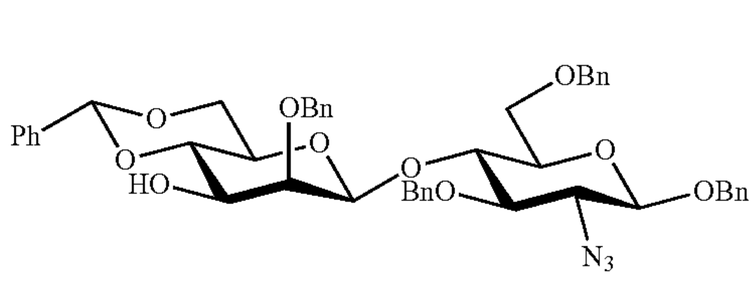
indicated that the free oligosaccharide was converted into a new oligosaccharide that was eluted earlier than the reducing sugar under the HPAEC condition (see general method). The product was purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound 5 (4.7 mg, 97%) as white solid after lyophilization with 5 mol. % of NaOH. 1H NMR (400 MHZ, D_2O) δ 6.01 (1H, d, J=7.3 Hz), 4.63 (1H, m), 4.36-4.47 (1H, m), 4.13-4.11 (1H, m), 3.99-3.89 (3H, m), 3.72-3.59 (3H, m), 3.58-3.54 (2H, m), 3.38-3.32 (2H, m), 1.99 (3H, d, J=1.7 Hz); ^{13}C NMR (100 MHz, D_2O) δ 168.70, 101.34, 99.88, 77.48, 75.88, 75.81, 72.57, 71.02, 70.50, 68.99, 66.54, 65.36, 63.25, 63.21, 61.82, 12.96; 31p NMR (146 MHZ, D_2O) δ 4.44. HRMS: $[M+H]^+$ calcd for $C_{14}H_{25}NO13P^+$, 446.1058; found, 446.1064.

Benzyl 2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (20)

[0185]



15

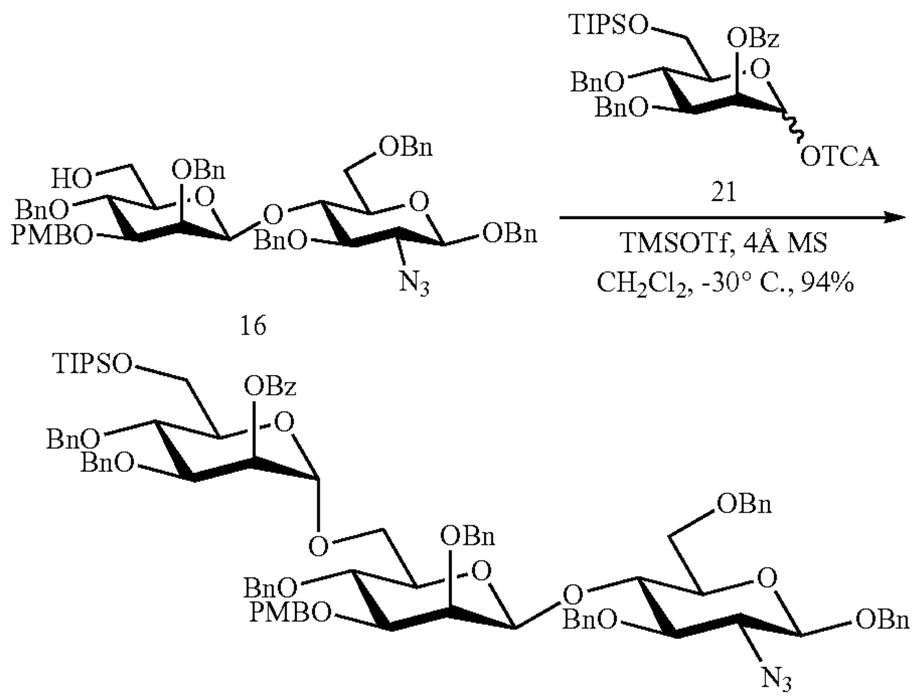


20

[0186] To a solution of 15 (454 mg, 0.485 mmol) in a mixture of CH_2Cl_2/H_2O (15 mL/1 mL) was added DDQ (252 mg, 1.11 mmol) at 0° C. After 30 min, the reaction mixture was warmed to room temperature and further stirred for 1 h. The reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$(aq.) and brine, and dried over Na_2SO_4. Concentration and purification by column chromatography on silica gel (hexanes/EtOAc=6:1~3:1) provided 20 (356 mg, 90%) as a white amorphous solid. R_f=0.25 (hexanes/EtOAc=3:1); Spectroscopic data were in agreement with literature values.[3] MALDI-TOF: $[M+Na]^+$ calcd for $C_{47}H_{49}N_3NaO_{10}$, 838.33; found, 838.47.

Benzyl 3,4-di-O-benzyl-2-O-benzoyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-0)-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (23)

[0187]

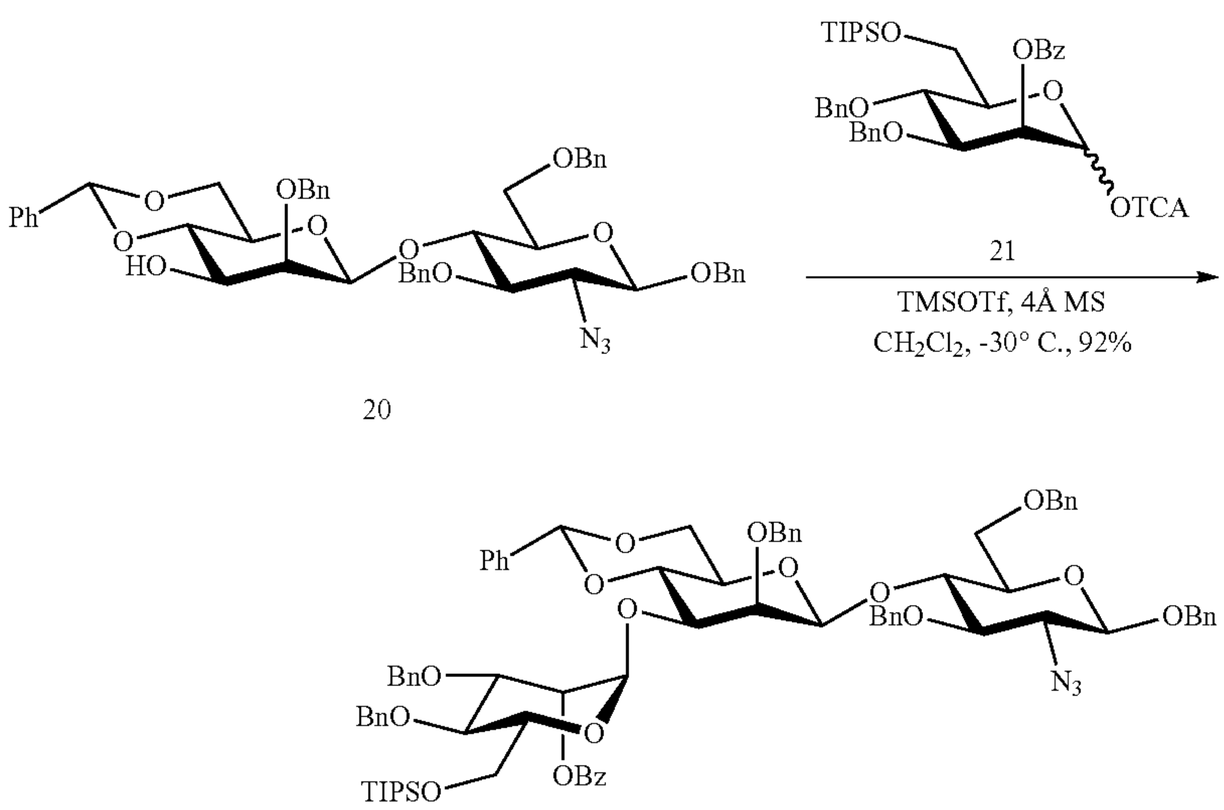


23

[0188] A mixture of trichloroacetimidate donor 21[4] (100 mg, 0.13 mmol), acceptor 16 (63 mg, 0.067 mmol) and activated 4 Å molecular sieves (200 mg) in anhydrous CH_2Cl_2 (2.0 mL) was stirred at room temperature under argon atmosphere for 1.5 h, and then cooled to −30° C. TMSOTf (1.0 μL, 5.5 μmol) was added. After stirring at −30° C. for 50 min, the mixture was quenched with triethylamine (20 μL) and filtered then concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes/EtOAc=10:1~4:1) to give product 23 (97.6 mg, 94%) as white foam. R_f=0.50 (hexanes/EtOAc=3:1); ^1H NMR (400 MHZ, $CDCl_3$) δ 8.10-8.08, 7.58, 7.45-7.13 (42H, m, Ar—H), 6.88-6.86 (2H, m, Ar—H), 5.66-5.64 (1H, m), 5.10 (1H, d, J=11.4 Hz, $PhCH_2$), 4.95-4.77 (7H, m, $PhCH_2$), 4.70-4.57 (5H, m), 4.52-4.39 (4H, m), 4.34-4.31 (2H, m), 4.08 (1H, dd, J=9.5 Hz, J=9.5 Hz), 4.02-3.97 (2H, m), 3.91-3.86 (2H, m), 3.82-3.76 (5H, m), 3.72-3.68 (3H, m), 3.63-3.59 (2H, m), 3.52 (1H, dd, J=8.3 Hz, J=9.7 Hz), 3.43-3.33 (4H, m), 1.09 (21H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.56, 159.28, 139.08, 138.90, 138.61, 138.51, 138.15, 138.01, 137.00, 132.97, 130.24, 130.16, 130.05, 129.29, 128.56, 128.46, 128.31, 128.21, 128.18, 128.14, 128.01, 127.93, 127.85, 127.78, 127.67, 127.61, 127.52, 127.43, 127.41, 127.36, 127.26, 113.85, 101.38, 100.73, 97.98, 82.63, 80.94, 78.23, 77.30, 75.04, 74.91, 74.85, 74.49, 74.44, 74.07, 73.87, 73.58, 72.75, 71.46, 71.31, 70.93, 69.05, 68.74, 66.73, 65.97, 62.30, 55.30, 18.10, 18.07, 12.06; MALDI-TOF: $[M+Na]^+$ calcd for $C_{91}H_{105}N_3NaO_{17}Si^+$, 1562.71; found, 1563.13.

Benzyl 3,4-di-O-benzyl-2-O-benzoyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (24)

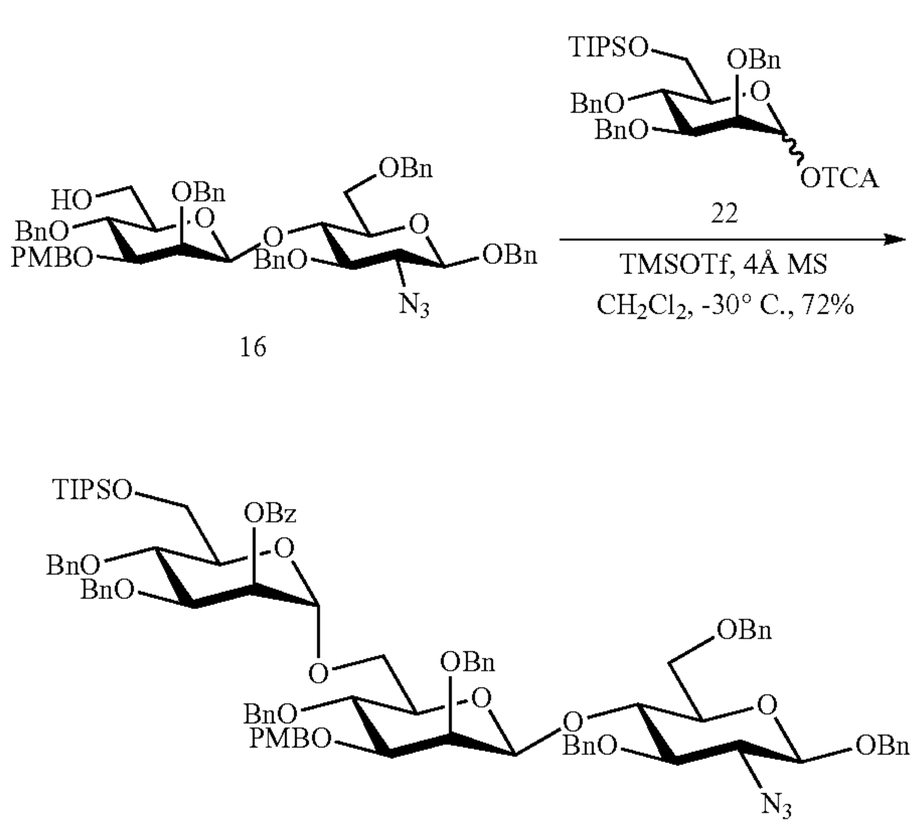
[0189]



[0190] A mixture of trichloroacetimidate donor 21[4] (187 mg, 0.245 mmol), acceptor 20 (100 mg, 0.123 mmol) and activated 4 Å molecular sieves (300 mg) in anhydrous CH_2Cl_2 (3.0 mL) was stirred at room temperature under argon atmosphere for 1.5 h, and then cooled to −30° C. TMSOTf (2.1 μL, 12.3 μmol) was added. After stirring at −30° C. for 1 h, the mixture was quenched with triethylamine (20 μL) and filtered then concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes/EtOAc=15:1~5:1) to give product 23 (159.4 mg, 92%) as white foam. R_f=0.30 (hexanes/EtOAc=8:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 8.01-7.98, 7.49, 7.36-7.09 (40H, m, Ar—H), 5.74 (1H, m), 5.45 (1H, s), 5.30 (1H, m), 4.96 (1H, m), 4.85-4.82 (2H, m), 4.71-4.48 (8H, m), 4.38-4.35 (2H, m), 4.22-4.20 (1H, m), 4.04-3.88 (6H, m), 3.83-3.80 (1H, m), 3.72-3.70 (1H, m), 3.68-3.63 (2H, m), 3.60-3.57 (1H, m), 3.51-3.48 (1H, m), 3.42-3.35 (2H, m), 3.28-3.21 (2H, m), 2.99-2.96 (1H, m), 1.00 (21H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.53, 138.85, 138.55, 138.27, 138.07, 137.66, 137.22, 136.87, 133.11, 130.00, 128.61, 128.51, 128.29, 128.17, 128.11, 128.05, 128.00, 127.97, 127.93, 127.84, 127.80, 127.72, 127.55, 125.90, 101.05, 101.01, 100.51, 98.76, 81.61, 78.79, 78.52, 78.16, 76.95, 75.66, 75.55, 75.23, 75.06, 74.98, 73.89, 73.69, 71.48, 70.90, 68.75, 68.38, 68.32, 67.03, 65.82, 62.76, 18.11, 12.07; MALDI-TOF: $[M+Na]^+$ calcd for $C_{91}H_{105}N_3NaO_{17}Si^+$, 1440.64; found, 1440.07.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (25)

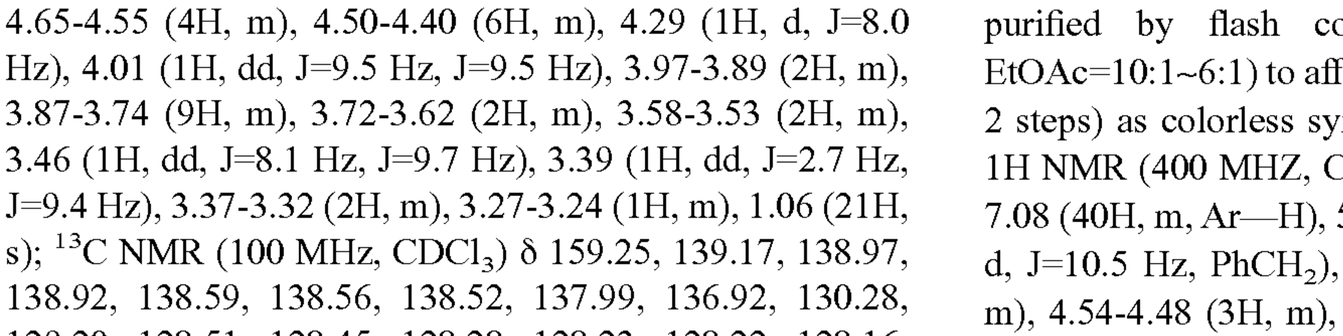
[0191]



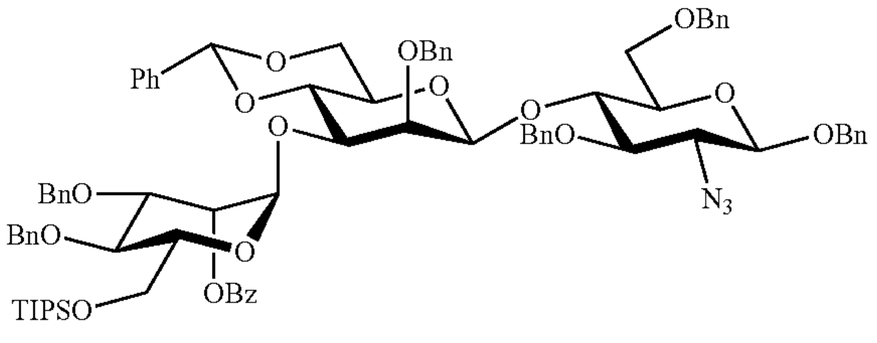
[0192] A mixture of trichloroacetimidate donor 22[5] (100 mg, 0.133 mmol), acceptor 16 (63 mg, 0.067 mmol) and activated 4 Å molecular sieves (200 mg) in anhydrous CH_2Cl_2 (2.0 mL) was stirred at room temperature under argon atmosphere for 1.5 h, and then cooled to −30° C. TMSOTf (1.0 μL, 5.5 μmol) was added. After stirring at −30° C. for 50 min, the mixture was quenched with triethylamine (20 μL) and filtered then concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes/EtOAc=10:1~4:1) to give the desired α-isomer 25 (74.3 mg, 72%) as white foam. R_f=0.40 (hexanes/EtOAc=4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.19 (42H, m, Ar—H), 6.87-6.85 (2H, m, Ar—H), 5.00 (1H, d, J=11.7 Hz, $PhCH_2$), 4.96-4.79 (7H, m, $PhCH_2$), 4.69 (1H, d, J=12.0 Hz, $PhCH_2$), 4.65-4.55 (4H, m), 4.50-4.40 (6H, m), 4.29 (1H, d, J=8.0 Hz), 4.01 (1H, dd, J=9.5 Hz, J=9.5 Hz), 3.97-3.89 (2H, m), 3.87-3.74 (9H, m), 3.72-3.62 (2H, m), 3.58-3.53 (2H, m), 3.46 (1H, dd, J=8.1 Hz, J=9.7 Hz), 3.39 (1H, dd, J=2.7 Hz, J=9.4 Hz), 3.37-3.32 (2H, m), 3.27-3.24 (1H, m), 1.06 (21H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.25, 139.17, 138.97, 138.92, 138.59, 138.56, 138.52, 137.99, 136.92, 130.28, 129.20, 128.51, 128.45, 128.28, 128.23, 128.22, 128.16, 128.14, 128.06, 127.92, 127.80, 127.77, 127.66, 127.61, 127.50, 127.46, 127.42, 127.32, 127.23, 127.11, 113.82, 101.61, 100.61, 98.19, 82.57, 80.75, 79.66, 77.60, 77.24, 75.42, 75.24, 74.92, 74.86, 74.75, 74.45, 74.28, 73.56, 73.41, 72.40, 71.52, 71.47, 70.90, 68.70, 66.12, 62.80, 55.28, 29.70, 18.05, 18.01, 12.04; MALDI-TOF: $[M+Na]^+$ calcd for $C_{91}H_{107}N_3NaO_{16}Si$, 1549.94; found, 1549.44.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (26)

[0193]



24

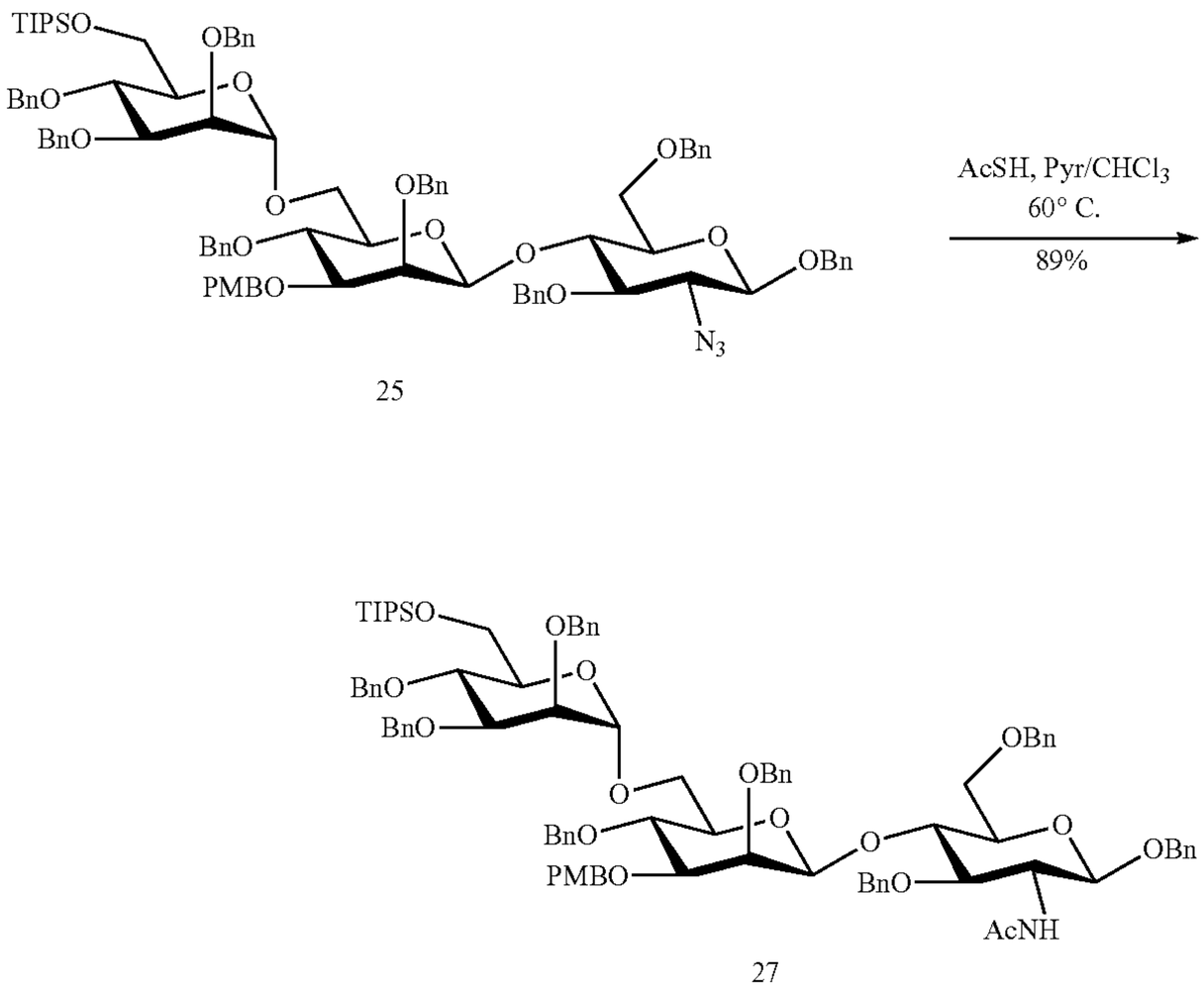


26

[0194] To a solution of compound 24 (90 mg, 0.0635 mmol) in MeOH (4.0 mL) was added sodium methoxide until pH=10, the solution was heated to 50° C. and stirred overnight. After the complete disappearance of the starting material, the solution was concentrated to dryness and dissolved in dry N,N-dimethylformamide (3.0 mL) and cooled to 0° C., sodium hydride (10.2 mg) and benzyl bromide (22.7 μL) were added successively, and the mixture was slowly warmed to room temperature. After the completion of the reaction as monitored by TLC, MeOH was added to quench the excess sodium hydride. The reaction was diluted with CH_2Cl_2, successively washed with H_2O and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash column chromatography (hexanes/EtOAc=10:1~6:1) to afford compound 26 (80.0 mg, 90% for 2 steps) as colorless syrup. R_f=0.20 (hexanes/EtOAc=6:1); 1H NMR (400 MHZ, $CDCl_3$) δ 7.48-7.46, 7.43-7.15, 7.10-7.08 (40H, m, Ar—H), 5.48 (1H, s), 5.34 (1H, m), 5.08 (1H, d, J=10.5 Hz, $PhCH_2$), 4.99-4.94 (2H, m), 4.78-4.61 (7H, m), 4.54-4.48 (3H, m), 4.43 (1H, d, J=12.4 Hz), 4.35-4.31 (2H, m), 4.07-3.98 (3H, m), 3.97-3.88 (4H, m), 3.85-3.82 (2H, m), 3.79-3.78 (1H, m), 3.73-3.68 (2H, m), 3.64-3.61 (1H, m), 3.53-3.46 (2H, m), 3.40-3.32 (2H, m), 3.10-3.03 (1H, m), 1.08-1.07 (21H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 138.92, 138.58, 138.56, 138.37, 138.34, 137.76, 137.49, 136.85, 129.22, 128.57, 128.48, 128.29, 128.27, 128.21, 128.13, 128.02, 127.97, 127.94, 127.92, 127.79, 127.72, 127.60, 127.47, 127.44, 127.38, 127.20, 126.16, 101.75, 100.90, 100.46, 98.47, 81.64, 79.72, 79.07, 78.87, 77.22, 76.58, 75.69, 75.63, 75.03, 74.97, 74.81, 74.61, 74.38, 73.60, 71.82, 70.86, 68.49, 68.33, 66.89, 65.78, 63.46, 29.71, 18.09, 18.07, 12.05; MALDI-TOF: $[M+Na]^+$ calcd for $C_{83}H_{97}N_3NaO_{15}Si$, 1426.66; found, 1426.03.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (27)

[0195]



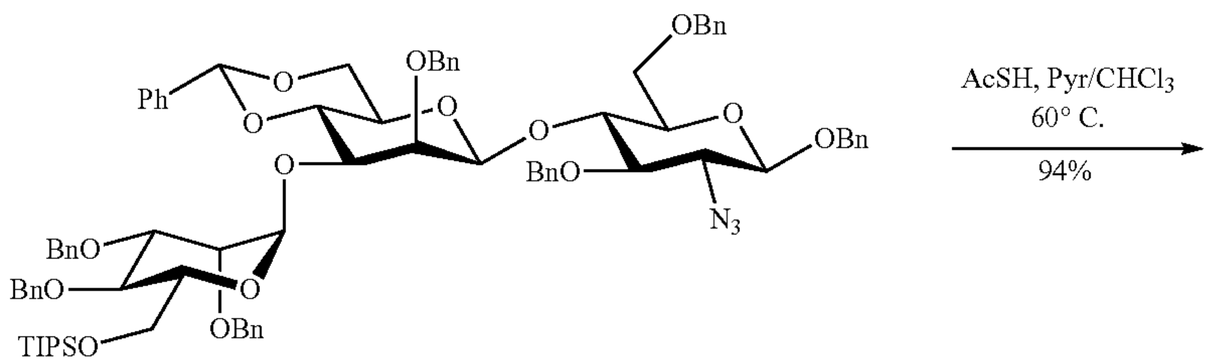
25

27

[0196] A solution of compound 25 (65.0 mg, 0.042 mmol) in a mixture of AcSH/pyridine/$CHCl_3$ (0.6 mL/0.4 mL/0.6 mL) was stirred at 60° ° C. for 18 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=8:1~3:1) to afford compound 27 (58.1 mg, 89%) as colorless syrup. R_f=0.30 (hexanes/EtOAc=3:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.41-7.18 (42H, m, Ar—H), 6.86-6.84 (2H, m, Ar—H), 5.33 (1H, d, J=7.9 Hz, NH), 4.95-4.83 (8H, m, PhCH/2), 4.63-4.53 (7H, m), 4.50-4.41 (6H, m), 4.06 (1H, dd, J=9.5 Hz, J=9.5 Hz), 4.03-3.97 (2H, m), 3.88-3.73 (11H, m), 3.66-3.63 (1H, m), 3.58-3.52 (4H, m), 3.44 (1H, dd, J=2.8 Hz, J=9.3 Hz), 3.33-3.29 (1H, m), 1.51 (3H, s), 1.04 (21H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 169.48, 158.75, 138.81, 138.64, 138.44, 138.19, 138.00, 137.63, 137.39, 129.76, 128.69, 127.94, 127.78, 127.75, 127.71, 127.67, 127.58, 127.55, 127.47, 127.28, 127.19, 127.16, 127.07, 127.02, 126.95, 126.80, 126.77, 126.59, 113.31, 100.63, 98.84, 97.51, 82.08, 79.65, 75.19, 74.80, 74.60, 74.54, 74.45, 74.36, 74.29, 74.04, 73.98, 73.11, 73.02, 72.72, 71.91, 71.32, 70.99, 70.23, 68.98, 65.87, 62.09, 54.77, 29.19, 22.69, 17.53, 17.49, 11.56; MALDI-TOF: $[M+Na]^+$ calcd for $C_{93}H_{111}NNaO_{17}Si$, 1565.98; found, 1565.46.

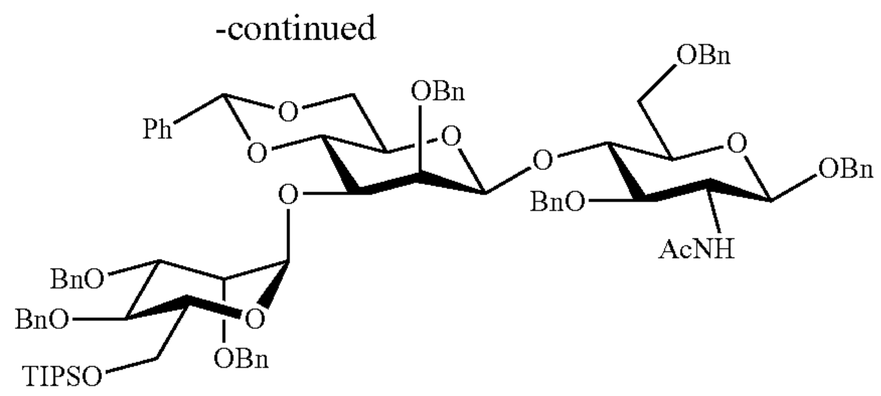
Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (28)

[0197]



26

-continued

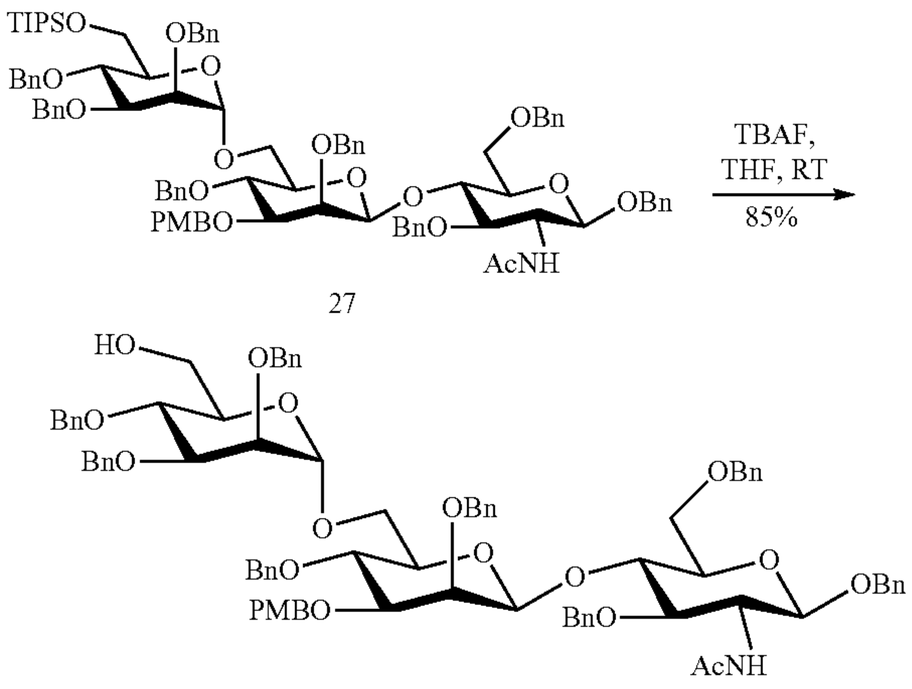


28

[0198] A solution of compound 26 (80.0 mg, 0.057 mmol) in a mixture of AcSH/pyridine/$CHCl_3$ (0.6 mL/0.4 mL/0.6 mL) was stirred at 60° C. for 18 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=4:1~2:1) to afford compound 27 (76.5 mg, 94%) as colorless syrup. R_f=0.25 (hexanes/EtOAc=2:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.50-7.48, 7.38-7.16, 7.11-7.09 (40H, m, Ar—H), 5.97 (1H, d, J=8.5 Hz, NH), 5.51 (1H, s), 5.36 (1H, m), 4.98-4.91 (3H, m), 4.81-4.77 (2H, m), 4.72 (1H, d, J=11.3 Hz, $PhCH_2$), 4.69-4.62 (3H, m), 4.60-4.57 (2H, m), 4.53-4.47 (4H, m), 4.37 (1H, d, J=12.4 Hz), 4.11-4.07 (1H, m), 4.06-3.82 (12H, m), 3.79-3.68 (3H, m), 3.59 (1H, dd, J=10.2 Hz, J=10.2 Hz), 3.19-3.13 (1H, m), 1.69 (3H, s), 1.08 (21H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.04, 138.83, 138.67, 138.50, 138.28, 138.02, 137.64, 137.40, 129.27, 128.46, 128.35, 128.32, 128.28, 128.21, 128.07, 127.93, 127.86, 127.83, 127.80, 127.67, 127.59, 127.52, 127.49, 127.47, 127.44, 127.26, 126.17, 101.82, 101.52, 99.26, 98.47, 79.71, 79.00, 78.91, 77.22, 75.93, 75.58, 75.28, 75.02, 74.69, 74.59, 74.49, 73.46, 72.94, 71.81, 70.54, 69.56, 68.58, 66.90, 63.44, 53.16, 23.13, 18.09, 18.07, 12.03; MALDI-TOF: $[M+Na]^+$ calcd for $C_{85}H_{101}NNaO_{16}Si$, 1442.68; found, 1442.29.

Benzyl 2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (29)

[0199]



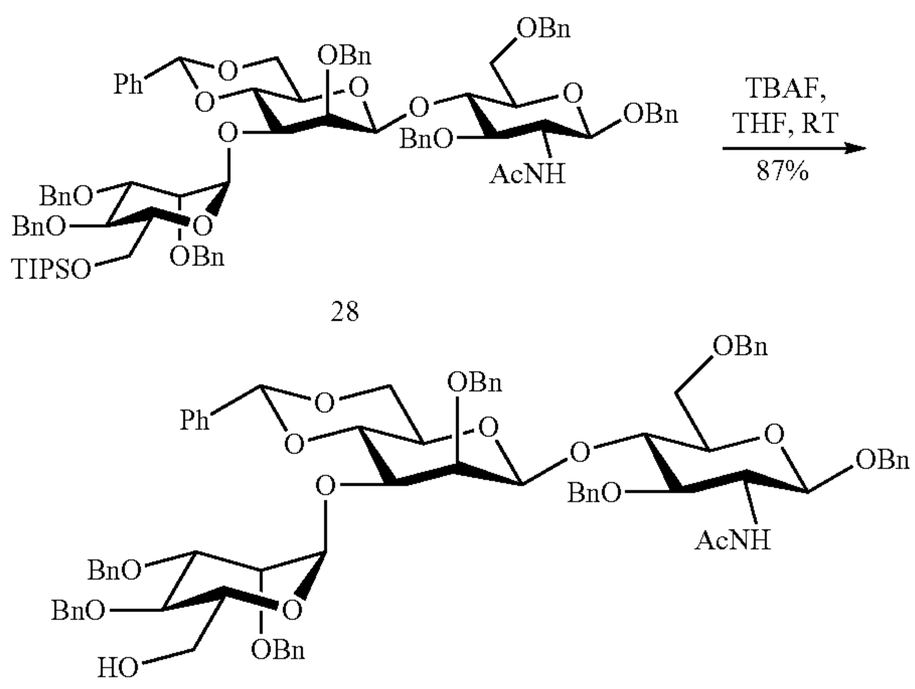
27

29

[0200] To a solution of compound 27 (65.0 mg, 0.042 mmol) in THF (2.0 mL) was added TBAF (1 M in THF, 210 μL), and the mixture was stirred at room temperature for 20 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=5:1~1:2) to afford compound 29 (49.0 mg, 85%) as colorless syrup. R_f=0.20 (hexanes/EtOAc=1:1); 1H NMR (400 MHZ, $CDCl_3$) δ 7.43-7.41, 7.36-7.21 (42H, m, Ar—H), 6.89-6.87 (2H, m, Ar—H), 5.41 (1H, d, J=8.0 Hz, NH), 5.07 (1H, m), 4.99-4.85 (7H, m, $PhCH_2$), 4.64-4.44 (12H, m), 4.17 (1H, dd, J=7.4 Hz, J=7.4 Hz), 4.08 (1H, dd, J=7.2 Hz, J=7.2 Hz), 3.96 (1H, dd, J=9.4 Hz, J=9.4 Hz), 3.89-3.80 (6H, m), 3.77-3.55 (9H, m), 3.47 (1H, dd, J=2.9 Hz, J=9.2 Hz), 3.39-3.35 (1H, m), 2.59 (1H, m), 1.56 (3H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 170.46, 159.30, 139.14, 138.79, 138.61, 138.49, 138.36, 138.00, 137.84, 130.17, 129.24, 128.47, 128.40, 128.35, 128.31, 128.29, 128.27, 128.24, 128.11, 127.85, 127.84, 127.79, 127.76, 127.72, 127.65, 127.61, 127.56, 127.53, 127.40, 127.34, 113.86, 101.18, 99.24, 97.88, 82.41, 79.89, 75.59, 75.30, 75.19, 75.05, 74.99, 74.76, 74.69, 73.62, 73.56, 72.73, 72.33, 71.86, 71.49, 70.74, 69.46, 67.01, 61.99, 55.30, 23.21; MALDI-TOF: $[M+Na]^+$ calcd for $C_{84}H_{91}NNaO_{17}$, 1408.62; found, 1408.27.

Benzyl 2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (30)

[0201]



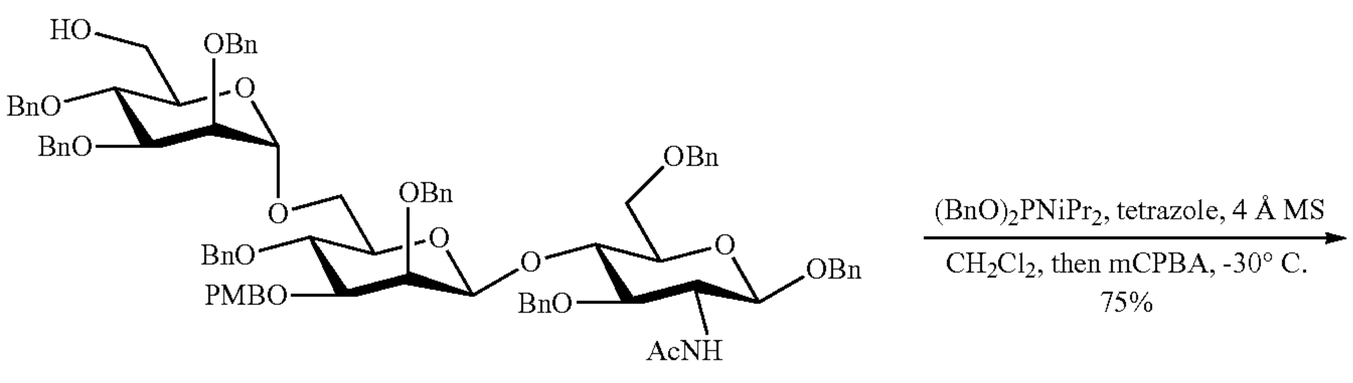
28

30

[0202] To a solution of compound 28 (76.5 mg, 0.054 mmol) in THF (2.0 mL) was added TBAF (1 M in THF, 269 μL), and the mixture was stirred at room temperature for 20 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=3:1~2:3) to afford compound 30 (59.3 mg, 87%) as colorless syrup. R_f=0.20 (hexanes/EtOAc=1:1); 1H NMR (400 MHZ, $CDCl_3$) δ 7.52-7.49, 7.41-7.16, 7.10-7.08 (40H, m, Ar—H), 5.78 (1H, d, J=8.0 Hz, NH), 5.52 (1H, s), 5.35 (1H, m), 5.01-4.91 (4H, m), 4.81-4.77 (2H, m), 4.78 (2H, m), 4.69-4.60 (5H, m), 4.53-4.47 (4H, m), 4.44-4.40 (1H, m), 4.17-3.95 (5H, m), 3.90-3.74 (7H, m), 3.71-3.58 (5H, m), 3.22-3.16 (1H, m), 1.77 (3H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 170.19, 138.86, 138.54, 138.34, 138.06, 137.97, 137.58, 137.45, 129.35, 128.55, 128.38, 128.34, 128.21, 128.19, 127.98, 127.94, 127.88, 127.82, 127.77, 127.71, 127.57, 127.54, 127.50, 126.19, 101.89, 101.55, 99.19, 98.81, 79.64, 78.94, 78.56, 77.86, 75.64, 75.46, 75.15, 74.78, 74.43, 73.70, 73.57, 72.92, 72.16, 71.89, 70.77, 69.11, 68.61, 66.98, 62.52, 55.15, 23.32; MALDI-TOF: $[M+Na]^+$ calcd for $C_{76}H_{81}NNaO_{16}^+$, 1286.54; found, 1286.20.

2,3,4-tri-O-benzyl-6-O-dibenzylphosphonato-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (31)

[0203]

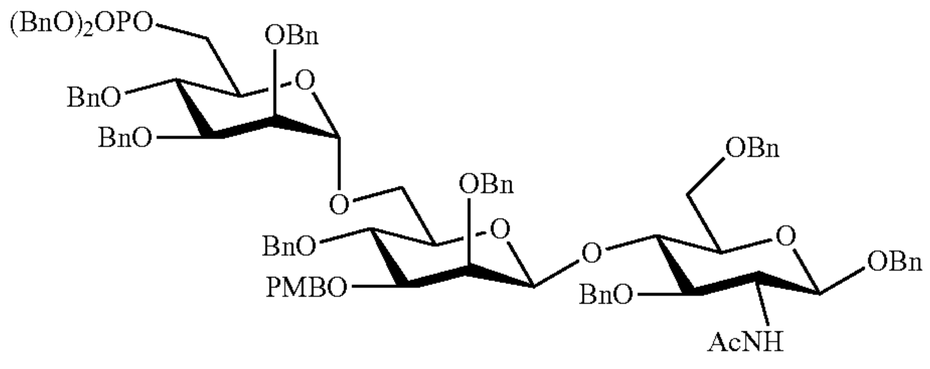


29

$(BnO)_2PNiPr_2$, tetrazole, 4 Å MS

CH_2Cl_2, then mCPBA, -30° C.

75%

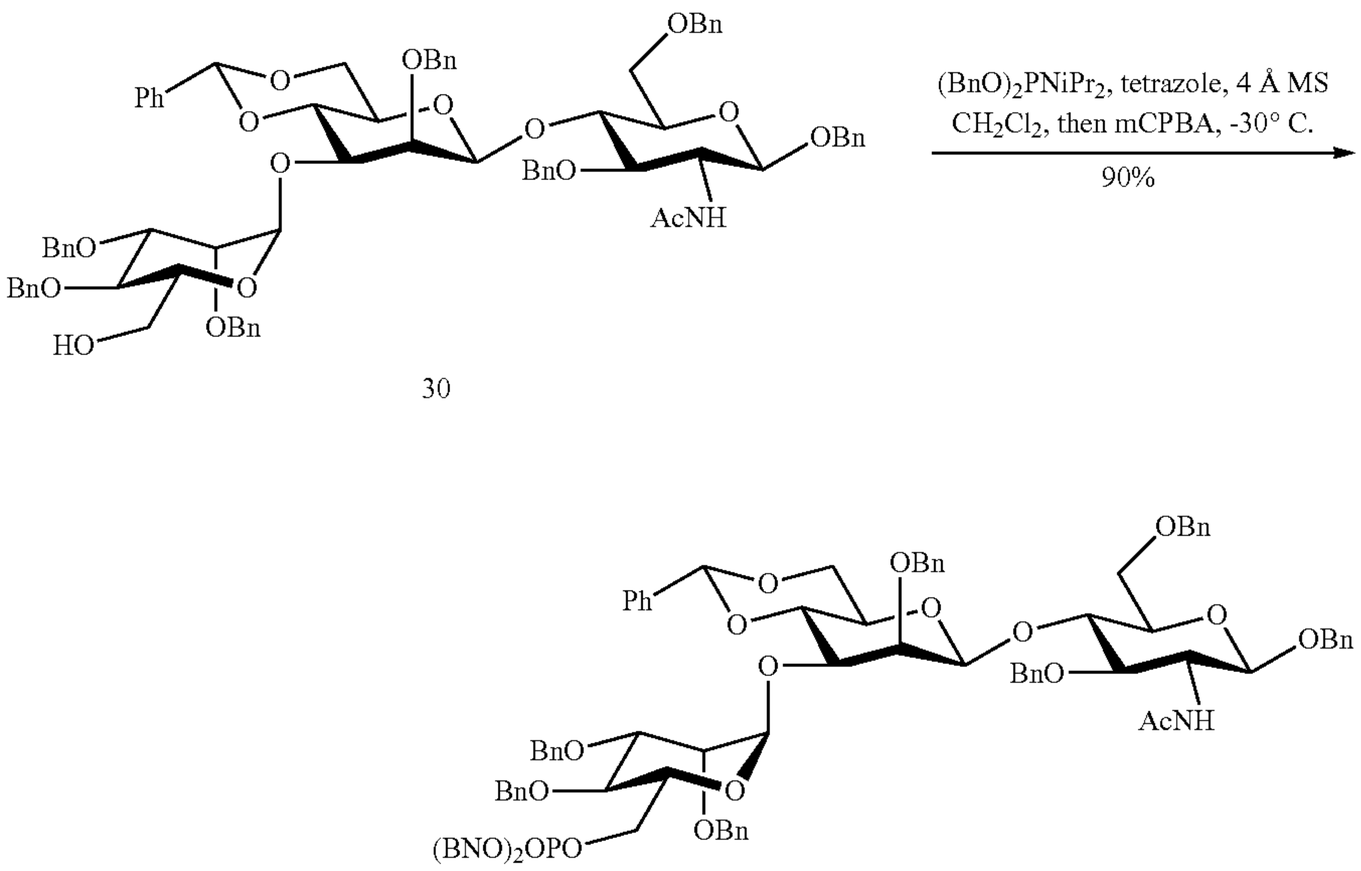


31

[0204] To a solution of compound 29 (38.0 mg, 0.027 mmol) in anhydrous CH_2Cl_2 (1.0 mL) was added activated 4 Å molecular sieves (100 mg) and tetrazole (0.45 M in MeCN, 305 μL) and the mixture was stirred at room temperature for 1.5 h before $(BnO)_2PNiPr_2$ (37.4 μL) was added. The resulting mixture was further stirred overnight under argon atmosphere at room temperature until the complete disappearance of the starting material. Then the reaction was cooled to −30° C., and mCPBA (77 wt %, 32.5 mg) was added, the reaction mixture was stirred at this temperature for 1 h and then filtered through a Celite pad. The filtrate was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (aq.), dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc=4:1~2:3) to give compound 31 (33.9 mg, 75%) as colorless syrup. R_f=0.20 (hexanes/EtOAc=1:1); ^1H NMR (400 MHz, $CDCl_3$) δ 7.40-7.38, 7.32-7.15 (52H, m, Ar—H), 6.86-6.84 (2H, m, Ar—H), 5.30 (1H, d, J=7.5 Hz, NH), 5.06-4.88 (8H, m), 4.85-4.82 (3H, m), 4.64-4.36 (12H, m), 4.11 (1H, dd, J=8.2 Hz, J=8.2 Hz), 4.05-3.96 (3H, m), 3.86-3.70 (11H, m), 3.62-3.56 (2H, m), 3.45 (1H, dd, J=2.9 Hz, J=9.3 Hz), 3.40-3.33 (2H, m), 1.53 (3H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 170.27, 159.24, 139.14, 138.66, 138.62, 138.46, 138.40, 138.17, 137.92, 136.19, 136.12, 136.07, 136.00, 130.22, 129.20, 128.45, 128.43, 128.37, 128.34, 128.27, 128.24, 128.20, 128.18, 128.14, 128.02, 127.90, 127.81, 127.70, 127.65, 127.61, 127.51, 127.47, 127.39, 113.81, 100.75, 99.30, 97.91, 82.53, 80.11, 77.77, 77.25, 77.15, 75.36, 75.21, 75.12, 75.01, 74.93, 74.83, 74.74, 74.53, 74.37, 73.97, 73.55, 72.57, 71.92, 71.42, 70.89, 70.77, 69.38, 69.18, 69.13, 69.07, 69.01, 66.74, 66.46, 56.21, 55.29, 29.72, 23.27; 31P NMR (146 MHz, $CDCl_3$) δ −1.24; MALDI-TOF: $[M+Na]^+$ calcd for $C_{98}H_{104}NNaO_{20}P^+$, 1668.68; found, 1669.05.

Benzyl 2,3,4-tri-O-benzyl-6-O-dibenzylphospho-nato-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-acet-amido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (32)

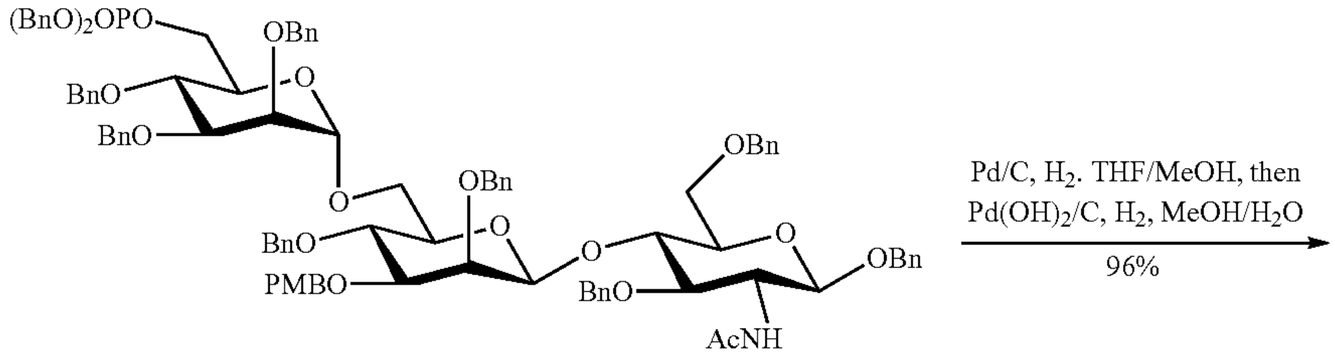
[0205]



[0206] To a solution of compound 30 (58.0 mg, 0.046 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added activated 4 Å molecular sieves (200 mg) and tetrazole (0.45 M in MeCN, 511 μL) and the mixture was stirred at room temperature for 1.5 h before $(BnO)_2PNiPr_2$ (62.5 μL) was added. The resulting mixture was further stirred overnight under argon atmosphere at room temperature until the complete disappearance of the starting material. Then the reaction was cooled to −30° C., and mCPBA (77 wt %, 54.4 mg) was added, the reaction mixture was stirred at this temperature for 1 h and then filtered through a Celite pad. The filtrate was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$(aq.), dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc=4:1~2:3) to give compound 32 (63.2 mg, 90%) as colorless syrup. R_f=0.20 (hexanes/EtOAc=1:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.49-7.46, 7.40-7.17, 7.14-7.10, 7.04-7.02 (50H, m, Ar—H), 5.83 (1H, d, J=8.1 Hz, NH), 5.49 (1H, s), 5.32 (1H, m), 5.10-5.00 (4H, m), 4.99-4.87 (4H, m), 4.76-4.72 (2H, m), 4.65-4.58 (5H, m), 4.51-4.45 (3H, m), 4.43-4.40 (1H, m), 4.37-4.42 (3H, m), 4.16-4.04 (2H, m), 4.01-3.93 (3H, m), 3.87-3.73 (6H, m), 3.70-3.64 (2H, m), 3.57 (1H, dd, J=10.3 Hz, J=10.3 Hz), 3.11-3.04 (1H, m), 1.76 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.20, 138.83, 138.43, 138.27, 138.09, 137.98, 137.62, 137.49, 135.96, 135.90, 129.31, 128.57, 128.53, 128.50, 128.46, 128.42, 128.35, 128.33, 128.20, 128.15, 128.03, 127.94, 127.91, 127.87, 127.78, 127.73, 127.65, 127.53, 127.47, 127.42, 126.20, 101.92, 101.24, 99.18, 98.64, 79.59, 78.94, 78.59, 75.64, 75.57, 75.25, 75.05, 74.32, 74.05, 73.51, 73.43, 71.98, 71.78, 70.68, 69.41, 69.35, 69.24, 69.19, 68.61, 66.74, 54.76, 23.29; 31P NMR (146 MHz, $CDCl_3$) δ −1.08; MALDI-TOF: $[M+Na]^+$ calcd for $C_{90}H_{94}NNaO_{19}P^+$, 1546.60; found, 1546.01.

6-O-phosphonato-α-D-mannopyranosyl-(1→6)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-αβ-D-glucopyranoside (33)

[0207]



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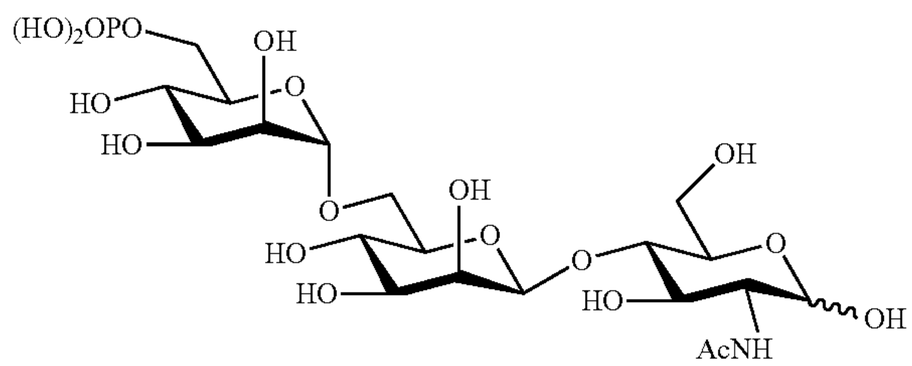
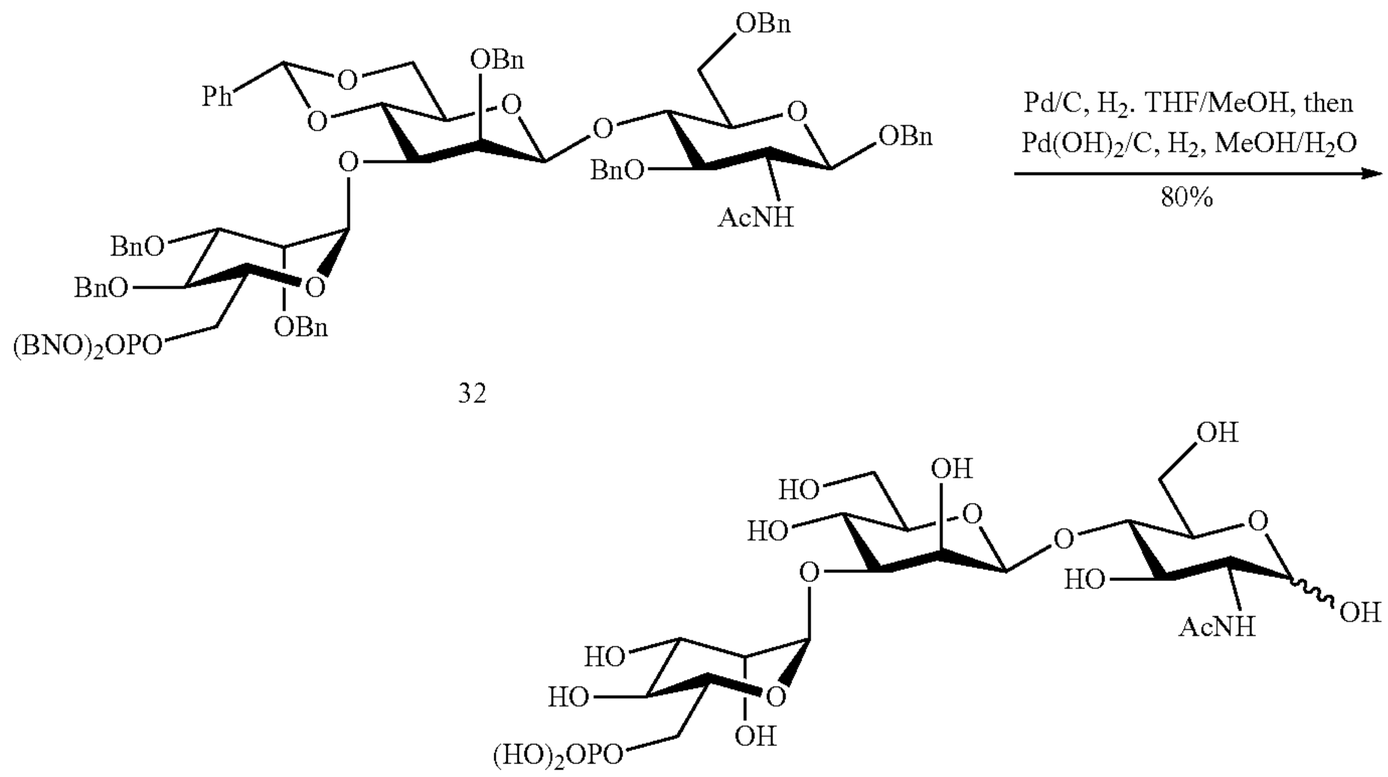


33

[0208] A mixture of compound 31 (60.2 mg, 0.036 mmol) and Pd/C (10 wt. % loading, 30 mg) in MeOH (2.0 mL) and THF (2.0 mL) was stirred under H_2 atmosphere for 21 h. The reaction mixture was filtered through a Celite pad, then concentrated to dryness. The mixture of the residue and $Pd(OH)_2/C$ (20 wt. % loading, 40 mg) in MeOH (2.5 mL) and H_2O (2.5 mL) was stirred under H_2 atmosphere for further 21 h. The reaction mixture was filtered through a Celite pad. The filtrate was concentrated to dryness then dissolved in H_2O and lyophilized. The crude product was purified on a Sephadex G-10 column by elution with H_2O. Fractions containing the product were pooled and lyophilized to give compound 33 (22.0 mg, 96%) as white solid. R_f=0.40 (n-BuOH/EtOH/H_2O/AcOH=1:1:1:0.05); ^1H NMR (400 MHZ, D_2O) δ 5.14 (0.63H, d, J=3.2 Hz), 4.84 (1.03H, m), 4.65 (0.39H, m), 4.03-3.98 (3.24H, m), 3.90-3.79 (5.69H, m), 3.75-3.62 (6.60H, m), 3.61-3.53 (4.03H, m), 1.99 (3H, s); ^{13}C NMR (100 MHZ, D_2O) δ 174.92, 174.62, 100.55, 100.51, 99.88, 99.84, 94.91, 90.48, 80.28, 79.95, 74.45, 74.30, 72.78, 72.30, 71.88, 71.80, 70.51, 70.46, 70.19, 70.13, 69.93, 69.89, 69.08, 66.70, 66.27, 66.22, 63.59, 60.29, 60.17, 56.01, 53.61, 22.30, 21.98; 31P NMR (146 MHZ, D_2O) δ 1.91 (overlapped signals); HRMS: $[M+H]^+$ calcd for $C_{20}H_{37}NO_{19}P^+$, 626.1692; found, 626.1690.

6-O-phosphonato-α-D-mannopyranosyl-(1→3)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-αβ-D-glucopyranoside (34)

[0209]



32

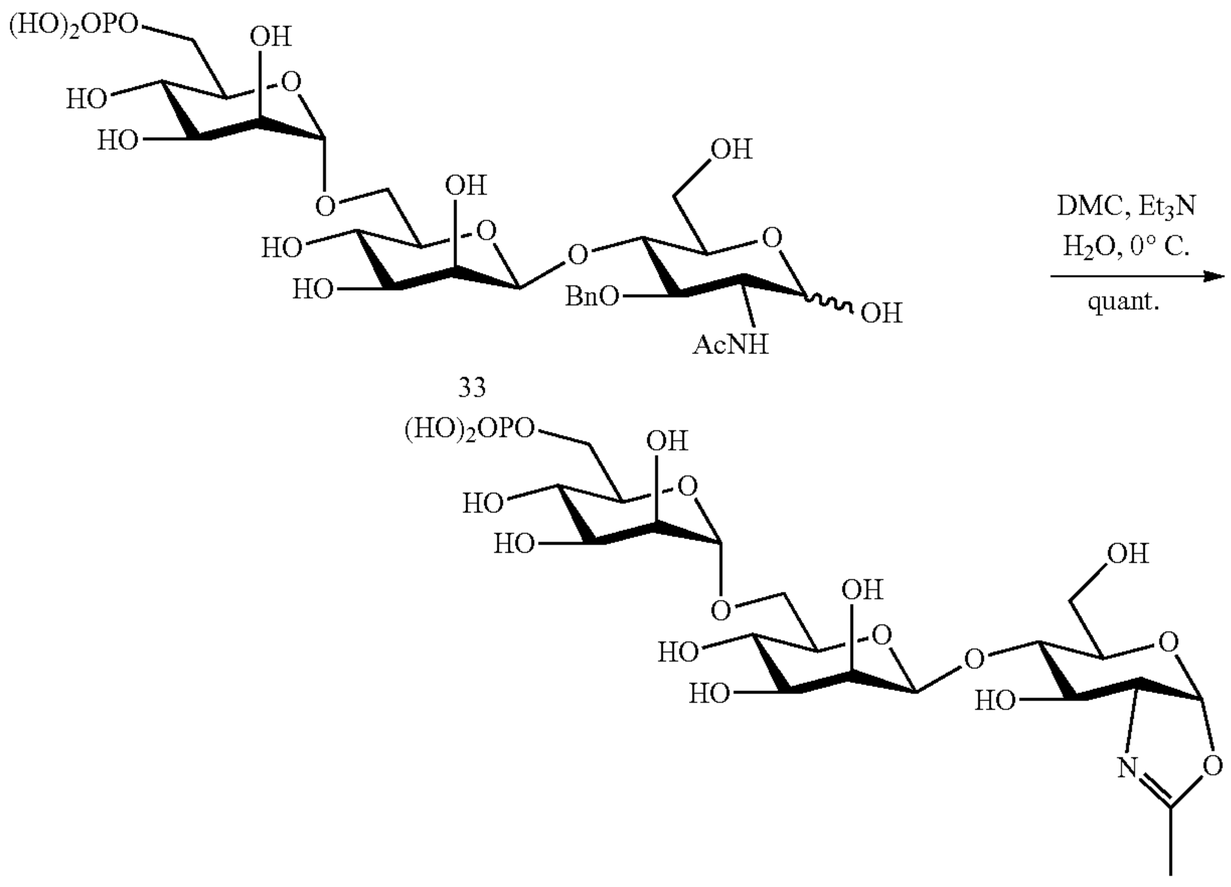
34

[0210] A mixture of compound 32 (53.0 mg, 0.034 mmol) and Pd/C (10 wt. % loading, 30 mg) in MeOH (2.0 mL) and THF (2.0 mL) was stirred under H_2 atmosphere for 21 h. The reaction mixture was filtered through a Celite pad, then concentrated to dryness. The mixture of the residue and $Pd(OH)_2/C$ (20 wt. % loading, 30 mg) in MeOH (2.5 mL) and H_2O (2.5 mL) was stirred under H_2 atmosphere for further 21 h. The reaction mixture was filtered through a Celite pad. The filtrate was concentrated to dryness then dissolved in H_2O and lyophilized. The crude product was purified on a Sephadex G-10 column by elution with H_2O. Fractions containing the product were pooled and lyophilized to give compound 34 (17.4 mg, 80%) as white solid. R_f=0.35 (n-BuOH/EtOH/H_2O/AcOH=1:1:1:0.05); ^1H NMR

(400 MHZ, D_2O) δ 5.14 (0.60H, d, J=3.4 Hz), 5.06-5.05 (0.98H, m), 4.66-4.64 (0.51H, m), 4.15 (1.06H, m), 4.07-3.96 (3.20H, m), 3.89-3.80 (5.62H, m), 3.76-3.59 (7.68H, m), 3.53-3.49 (0.45H, m), 3.42-3.39 (0.96H, m), 1.98 (3H, s); ^{13}C NMR (100 MHZ, D_2O) δ 174.31, 174.02, 102.02, 101.98, 99.44, 94.51, 90.11, 80.15, 80.01, 78.87, 78.49, 75.66, 74.29, 72.05, 71.87, 69.85, 69.80, 69.74, 69.60, 68.70, 66.01, 65.59, 65.53, 63.59, 60.47, 59.87, 59.76, 55.71, 53.26, 21.78, 21.49; 31P NMR (146 MHz, D_2O) δ 1.71 (overlapped signals); HRMS: $[M+H]^+$ calcd for $C_{20}H_{37}NO_{19}P^+$, 626.1692; found, 626.1690.

2-Methyl-[6-O-phosphonato-α-D-mannopyranosyl-(1→6)-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline (6)

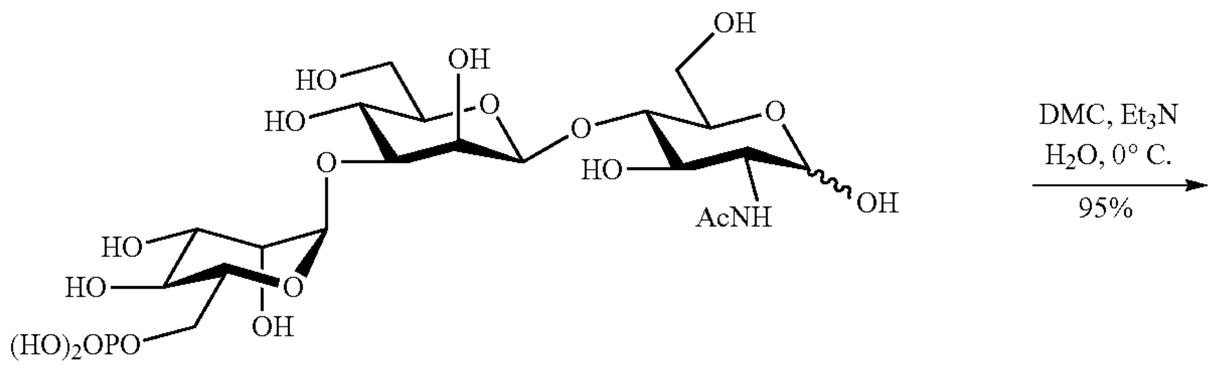
[0211]



[0212] To a solution of compound 33 (8.0 mg, 0.013 mmol) in H_2O (300 μL) were added Et_3N (72.0 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 43.4 mg) at 0° C. The reaction mixture was monitored by DIONEX HPAEC-PAD. After 2 h, the HPAEC analysis indicated that the free oligosaccharide was converted into a new oligosaccharide that was eluted earlier than the reducing sugar under the HPAEC condition (see general method). The product was purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound 6 (7.8 mg, quant.) as white solid after lyophilization with 5 mol. % of NaOH. 1H NMR (400 MHZ, D_2O) δ 6.02 (1H, d, J=7.3 Hz), 4.87 (1H, m), 4.84-4.81 (1H, m), 4.64-4.62 (1H, m), 4.31-4.30 (1H, m), 4.15-4.12 (1H, m), 4.01-3.95 (2H, m), 3.92-3.86 (4H, m), 3.81-3.78 (2H, m), 3.80-3.76 (1H, m), 3.74-3.73 (1H, m), 3.71-3.70 (1H, m), 3.68-3.65 (3H, m), 3.61-3.54 (3H, m), 3.48-3.44 (1H, m), 3.36-3.34 (1H, m), 1.99 (3H, d, J=1.8 Hz); ^{13}C NMR (100 MHZ, D_2O) δ 168.55, 101.49, 99.90, 99.83, 77.63, 74.51, 72.96, 72.25, 70.92, 70.48, 70.18, 70.00, 69.06, 66.48, 66.03, 65.81, 65.16, 62.59, 61.77, 12.96; 31P NMR (146 MHz, D_2O) δ 4.45; HRMS: $[M+H]^+$ calcd for $C_{20}H_{35}NO_{18}P^+$, 608.1586; found, 608.1598.

2-Methyl-[6-O-phosphonato-α-D-mannopyranosyl-(1→3)-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline (7)

[0213]



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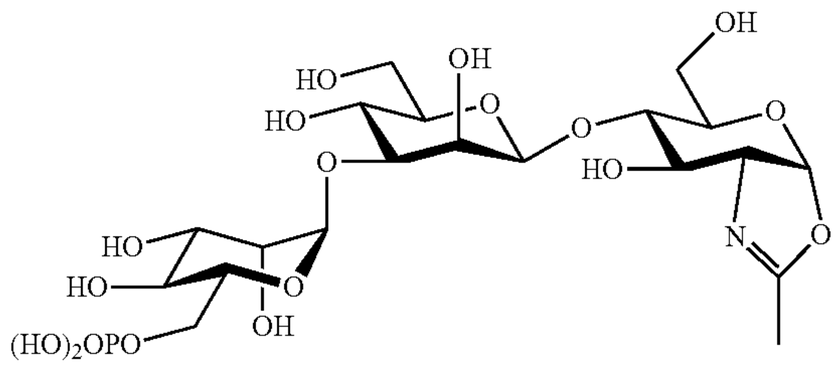
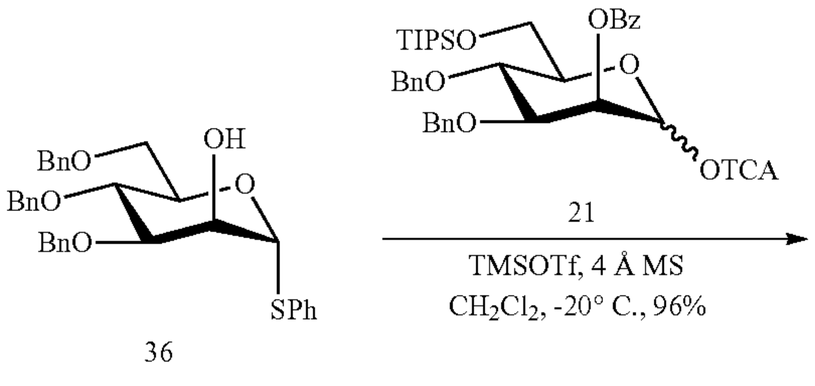


7

[0214] To a solution of compound 34 (8.0 mg, 0.013 mmol) in H_2O (300 μL) were added Et_3N (72.0 L) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 43.4 mg) at 0° C. The reaction mixture was monitored by DIONEX HPAEC-PAD. After 2 h, the HPAEC analysis indicated that the free oligosaccharide was converted into a new oligosaccharide that was eluted earlier than the reducing sugar under the HPAEC condition (see general method). The product was purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound 7 (7.4 mg, 95%) as white solid after lyophilization with 5 mol. % of NaOH. 1H NMR (400 MHZ, D_{20}) δ 6.00 (1H, d, J=7.3 Hz), 5.02 (1H, m), 4.30-4.28 (1H, m), 4.11-4.08 (1H, m), 4.03-4.02 (1H, m), 3.98-3.94 (2H, m), 3.88-3.81 (4H, m), 3.78-3.76 (1H, m), 3.74-3.70 (2H, m), 3.68-3.64 (4H, m), 3.60-3.55 (2H, m), 3.37-3.33 (2H, m), 1.99 (3H, d, J=1.5 Hz); ^{13}C NMR (100 MHZ, D_{20}) δ 168.61, 102.51, 101.10, 99.94, 80.35, 77.67, 76.12, 72.82, 72.74, 70.97, 70.13, 70.00, 69.34, 66.19, 66.06, 65.20, 62.65, 62.61, 61.56, 61.04, 13.00; 31P NMR (146 MHz, D_2O) δ 4.45; HRMS: $[M+H]^+$ calcd for $C_{20}H_{35}NO_{18}P^+$, 608.1586; found, 608.1599.

Phenyl 3,4-di-O-benzyl-2-O-benzoyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-1-thio-α-D-mannopyranoside (37)

[0215]



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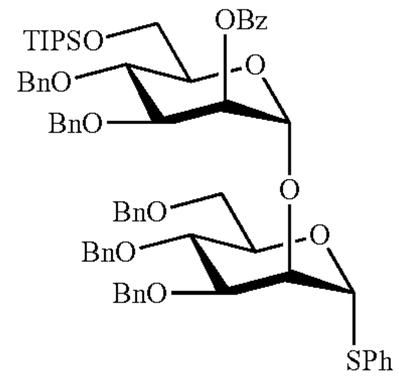
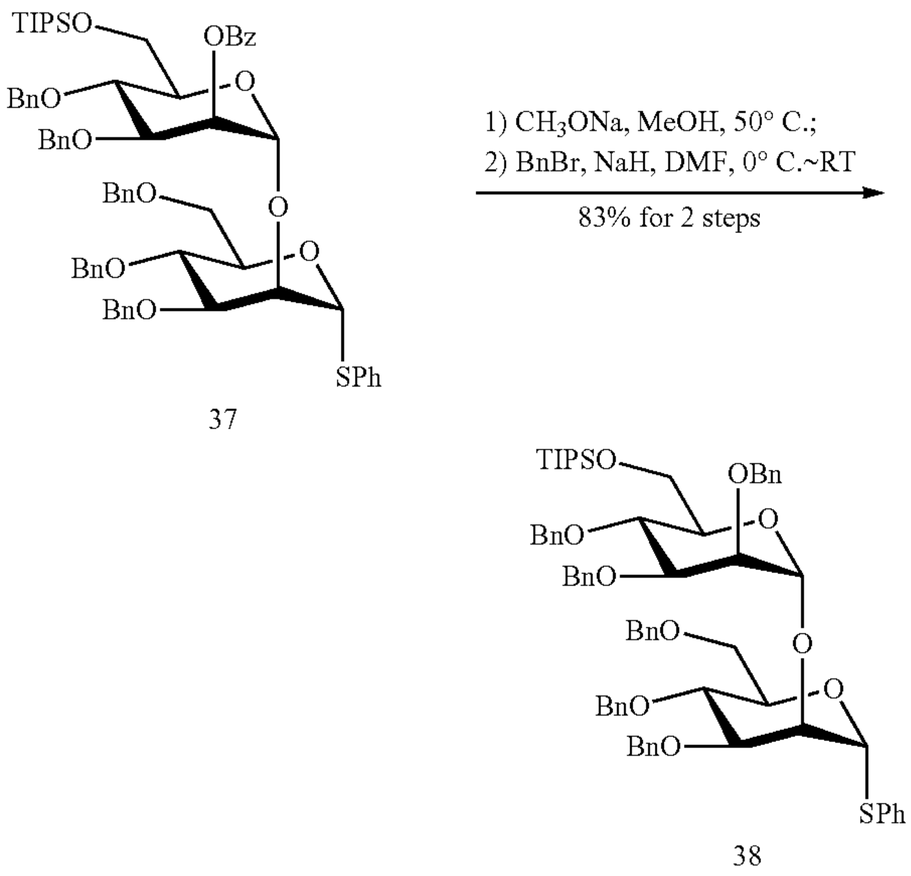


37

[0216] A mixture of trichloroacetimidate donor 21[4] (895 mg, 1.17 mmol), acceptor 36[5] (489 mg, 0.902 mmol) and activated 4 Å molecular sieves (1.0 g) in anhydrous CH_2Cl_2 (10 mL) was stirred at room temperature under argon atmosphere for 1.5 h, and then cooled to −20° C. TMSOTf (16.0 μL, 0.09 mmol) was added. After stirring at −20° ° C. for 0.5 h, the mixture was quenched with triethylamine (20 μL) and filtered then concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes/EtOAc=15:1~10:1) to give product 37 (995 mg, 96%) as colorless syrup. R_f=0.60 (hexanes/EtOAc=5:1); ^1H NMR (400 MHZ, $CDCl_3$) δ 8.20-8.17, 7.67-7.63, 7.54-7.51, 7.45, 7.41-7.26 (35H, m, Ar—H), 5.82 (1H, m), 5.65 (1H, d, J=1.5 Hz), 5.29 (1H, d, J=1.6 Hz), 4.99-4.93, 4.84-4.78, 4.75-4.65, 4.57-4.53 (10H, m, $PhCH_2$), 4.37-4.34 (2H, m), 4.21-4.11 (3H, m), 4.05-3.98 (2H, m), 3.92-3.79 (4H, m), 1.15-1.11 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.63, 138.81, 138.52, 138.46, 138.27, 138.00, 134.44, 133.11, 131.52, 130.16, 130.07, 129.04, 128.54, 128.43, 128.34, 128.32, 128.26, 128.10, 128.09, 128.00, 127.95, 127.83, 127.72, 127.59, 127.56, 127.51, 127.46, 127.41, 99.61, 87.42, 80.25, 78.38, 77.31, 75.92, 75.31, 74.81, 74.04, 73.46, 73.27, 73.04, 72.30, 71.89, 69.39, 69.27, 62.39, 18.13, 18.08, 12.07; MALDI-TOF: $[M+Na]^+$ calcd for $C_{69}H_{80}NaO_{11}SSi^+$, 1167.51; found, 1167.73.

Phenyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-1-thio-α-D-mannopyranoside (38)

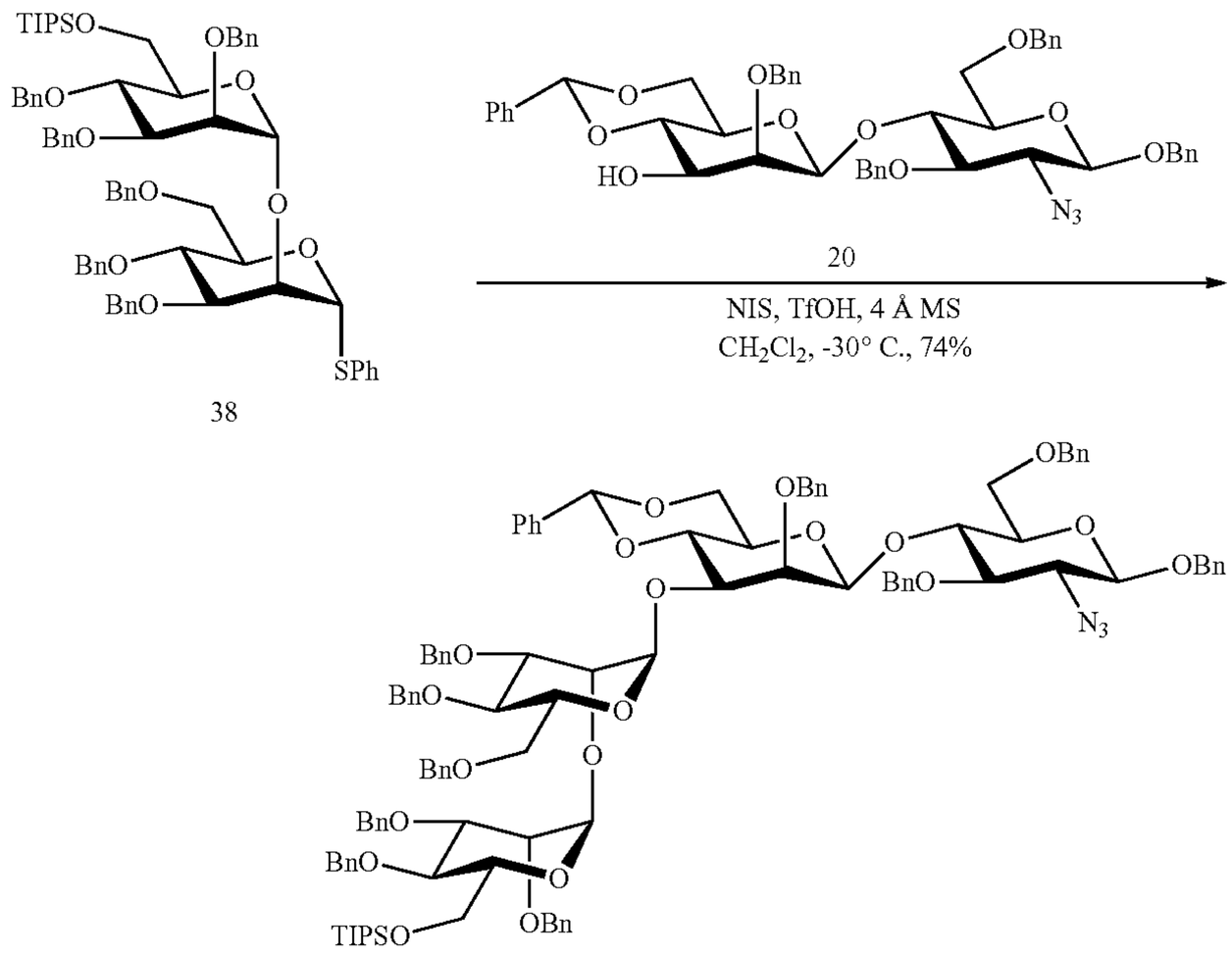
[0217]



[0218] To a solution of compound 37 (1.30 g, 1.136 mmol) in MeOH (12.0 mL) was added sodium methoxide until pH=10, the solution was heated to 50° C. and stirred overnight. After the complete disappearance of the starting material, the solution was concentrated to dryness and dissolved in dry N,N-dimethylformamide (10.0 mL) and cooled to 0° C., sodium hydride (78.4 mg) and benzyl bromide (225 μL) were added successively, and the mixture was slowly warmed to room temperature. After the completion of the reaction as monitored by TLC, MeOH was added to quench the excess sodium hydride. The reaction was diluted with CH_2Cl_2, successively washed with H_2O and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash column chromatography (hexanes/EtOAc=15:1~10:1) to afford compound 38 (1.059 g, 83% for 2 steps) as colorless syrup. R_f=0.60 (hexanes/EtOAc=8:1); 1H NMR (400 MHZ, $CDCl_3$) δ 7.51-7.48, 7.39-7.27 (35H, m, Ar—H), 5.60 (1H, d, J=1.4 Hz), 5.31 (1H, d, J=2.3 Hz), 4.96-4.90 (2H, m), 4.74-4.69 (3H, m), 4.67 (1H, m), 4.64-4.58 (2H, m), 4.58-4.49 (5H, m), 4.39 (1H, m), 4.32 (1H, m), 4.05 (1H, dd, J=9.4 Hz, J=9.4 Hz), 4.00-3.82 (7H, m), 3.77-3.71 (2H, m), 1.15-1.13 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.84, 138.78, 138.54, 138.51, 138.42, 137.91, 134.42, 131.20, 128.54, 128.98, 128.56, 128.44, 128.38, 128.33, 128.29, 128.27, 128.15, 128.12, 128.08, 128.03, 128.00, 127.98, 127.78, 127.73, 127.54, 127.42, 127.39, 127.22, 98.91, 87.31, 80.76, 79.81, 77.28, 75.25, 75.11, 75.06, 74.97, 74.76, 74.35, 74.10, 73.29, 72.94, 72.45, 72.30, 72.04, 69.28, 63.01, 18.09, 18.05, 12.06; MALDI-TOF: $[M+Na]^+$ calcd for $C_{69}H_{82}NaO_{10}SSi^+$, 1153.53; found, 1152.91.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (39)

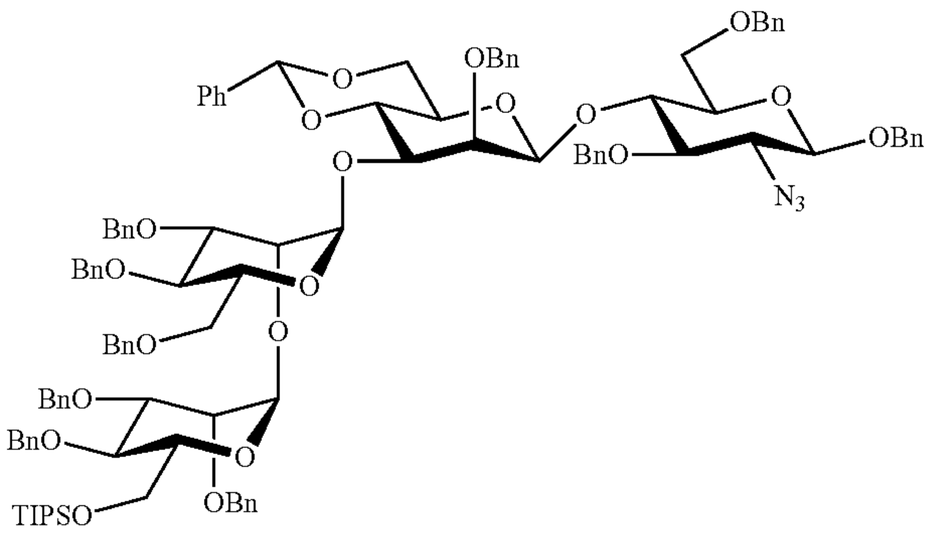
[0219]



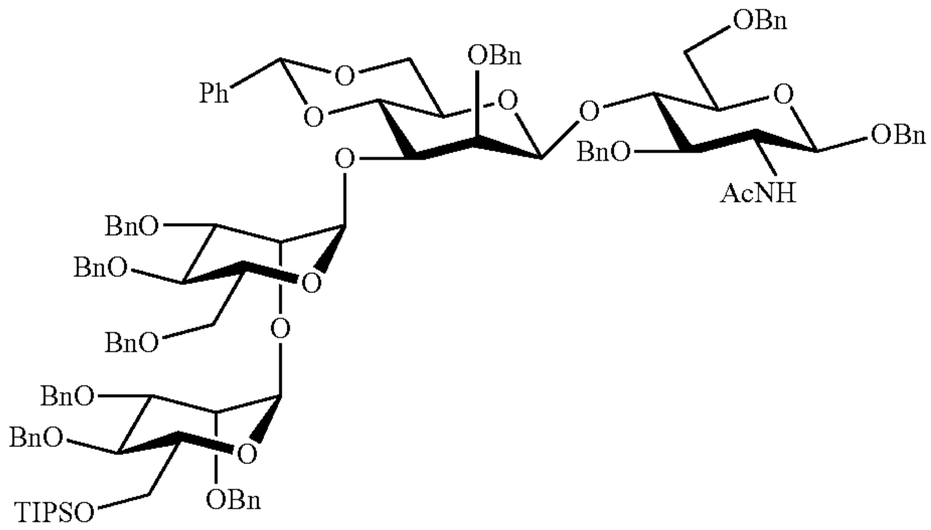
[0220] A mixture of compound 38 (180 mg, 0.159 mmol), acceptor 20 (100 mg, 0.123 mmol) and activated 4 Å molecular sieves (450 mg) in anhydrous CH_2Cl_2 (4.5 mL) was stirred at room temperature under argon atmosphere for 1.5 h, and then cooled to −30° C. N-iodosuccinimide (55.2 mg, 0.245 mmol) and TfOH (2.15 μL, 0.025 mmol) were successively added. After stirring at −30° C. for 2 h, the mixture was quenched with triethylamine (10 μL) and filtered then concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes/EtOAc=10:1~5:1) to give product 39 (166 mg, 74%) as colorless syrup. R_f=0.50 (hexanes/EtOAc=4:1); Spectroscopic data were in agreement with literature values.[7] MALDI-TOF: $[M+H]^+$ calcd for $C_{110}H_{126}N_3O_{20}Si^+$, 1836.87; found, 1836.44.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (40)

[0221]



39

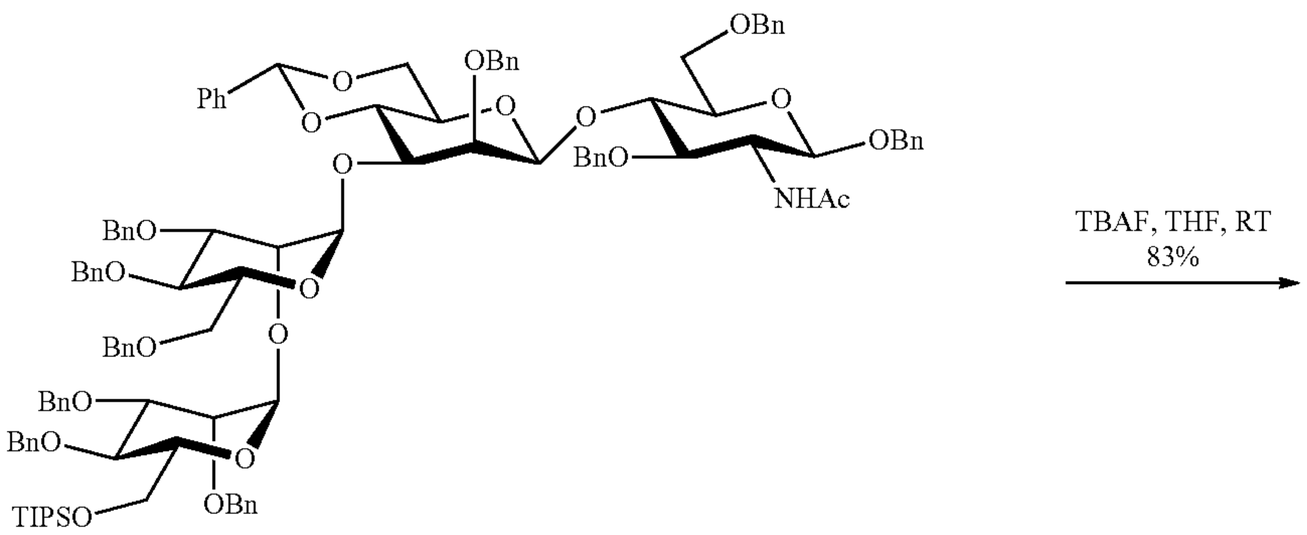


40

[0222] A solution of compound 39 (133.5 mg, 0.073 mmol) in a mixture of AcSH/pyridine/$CHCl_3$ (0.6 mL/0.4 mL/0.6 mL) was stirred at 60° C. for 18 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=4:1~1:1) to afford compound 40 (115.8 mg, 86%) as colorless syrup. R_f=0.30 (hexanes/EtOAc=2:1); ^1H NMR (400 MHZ, $CDCl_3$) δ 7.42-7.23 (54H, m), 7.03 (1H, t, J=7.7 Hz), 5.86 (1H, d, J=8.2 Hz), 5.51 (1H, s), 5.40 (1H, m), 5.29 (1H, m), 4.97-4.88 (4H, m), 4.86 (1H, d, J=4.2 Hz), 4.83 (2H, m), 4.68-4.38 (15H, m), 4.31 (1H, m), 4.19-4.01 (5H, m), 3.98-3.85 (5H, m), 3.82 (1H, m), 3.79-3.53 (10H, m), 3.50 (1H, m), 3.09 (1H, m), 1.75 (3H, s), 1.32-1.28 (3H, m), 1.08 (18H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 170.17, 139.18, 138.89, 138.78, 138.49, 138.46, 138.44, 138.20, 137.97, 137.89, 137.63, 137.16, 128.51, 128.47, 128.40, 128.37, 128.33, 128.28, 128.22, 128.16, 128.10, 128.05, 127.94, 127.90, 127.78, 127.74, 127.70, 127.57, 127.52, 127.34, 127.28, 125.75, 101.52, 101.40, 99.82, 99.20, 97.58, 79.74, 78.92, 78.64, 77.61, 77.27, 75.88, 75.32, 75.19, 75.01, 74.72, 74.57, 74.36, 74.30, 73.72, 73.44, 73.04, 72.26, 72.10, 71.53, 70.90, 70.67, 69.64, 69.26, 68.50, 67.01, 62.38, 60.42, 54.48, 29.73, 23.25, 18.12, 18.08, 12.10; MALDI-TOF: $[M+Na]^+$ calcd for $C_{112}H_{129}NNaO_{21}Si^+$, 1876.32; found, 1875.81.

Benzyl 2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-manno-pyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (41)

[0223]



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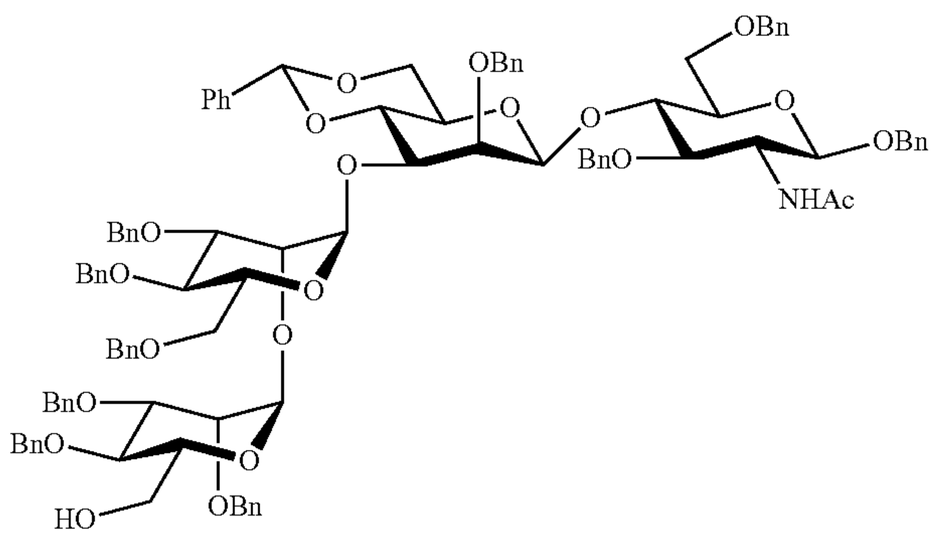
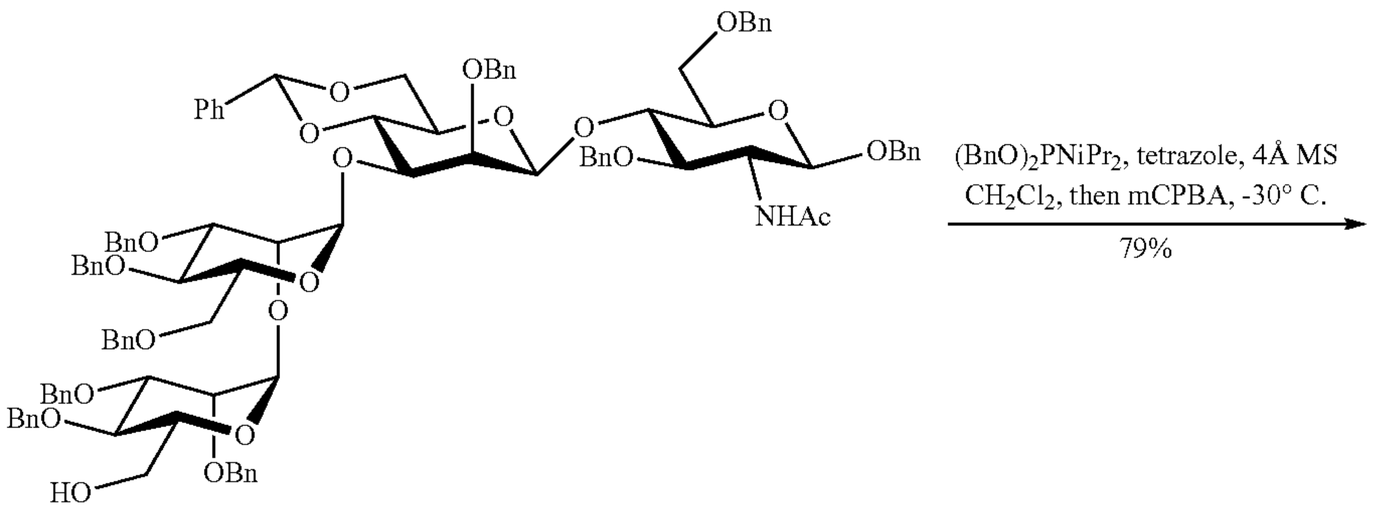


41

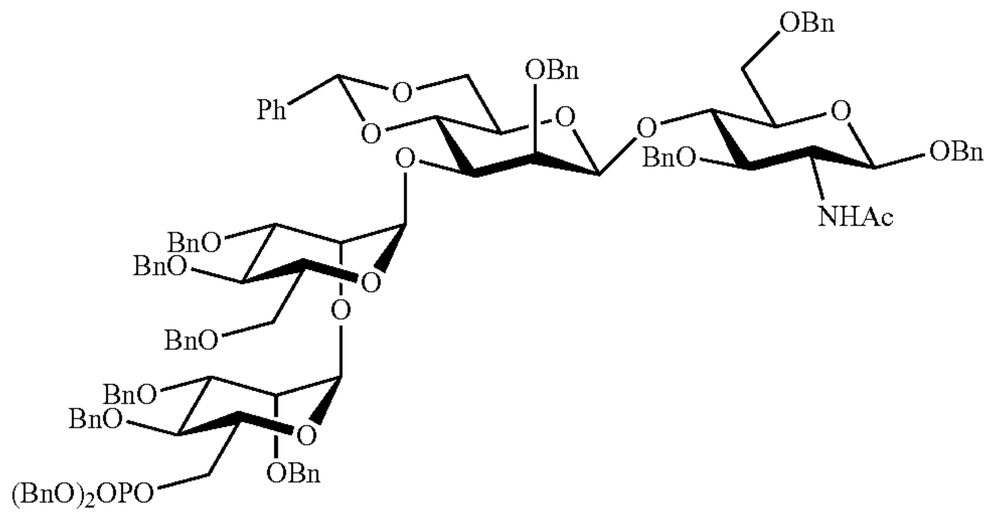
[0224] To a solution of compound 40 (115.8 mg, 0.063 mmol) in THF (2.0 mL) was added TBAF (1 M in THF, 313 μL), and the mixture was stirred at room temperature for 23 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=2:1~2:3) to afford compound 41 (88.0 mg, 83%) as colorless syrup. R_f=0.10 (hexanes/EtOAc=3:2); 1H NMR (400 MHZ, $CDCl_3$) δ 7.46-7.21 (55H, m), 5.78 (1H, d, J=8.0 Hz), 5.49 (1H, s), 5.25 (1H, d, J=1.5 Hz), 5.04 (1H, m), 4.96 (1H, d, J=6.7 Hz), 4.93-4.79 (6H, m), 4.65-4.48 (15H, m), 4.43 (1H, m), 4.10-4.04 (3H, m), 4.00-3.79 (9H, m), 3.78-3.69 (5H, m), 3.64-3.53 (5H, m), 3.29-3.26 (2H, m), 3.09 (1H, m), 1.75 (3H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 170.19, 138.82, 138.65, 138.60, 138.51, 138.45, 138.37, 138.20, 138.16, 137.88, 137.61, 137.25, 129.12, 128.56, 128.47, 128.44, 128.38, 128.35, 128.29, 128.24, 128.22, 128.18, 128.05, 127.99, 127.95, 127.91, 127.85, 127.82, 127.77, 127.73, 127.65, 127.52, 127.48, 126.08, 101.67, 101.44, 99.97, 99.58, 99.17, 79.58, 78.88, 78.67, 77.70, 77.26, 75.78, 75.60, 75.25, 75.10, 74.92, 74.84, 74.77, 74.43, 73.63, 73.50, 73.38, 72.83, 72.41, 72.32, 72.21, 70.72, 69.55, 69.07, 68.53, 66.93, 61.84, 54.91, 29.73, 23.31; MALDI-TOF: $[M+Na]^+$ calcd for $C_{103}H_{109}NNaO_{21}^+$, 1719.98; found, 1719.60.

Benzyl 2,3,4-tri-O-benzyl-6-O-dibenzylphospho-nato-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (42)

[0225]



41

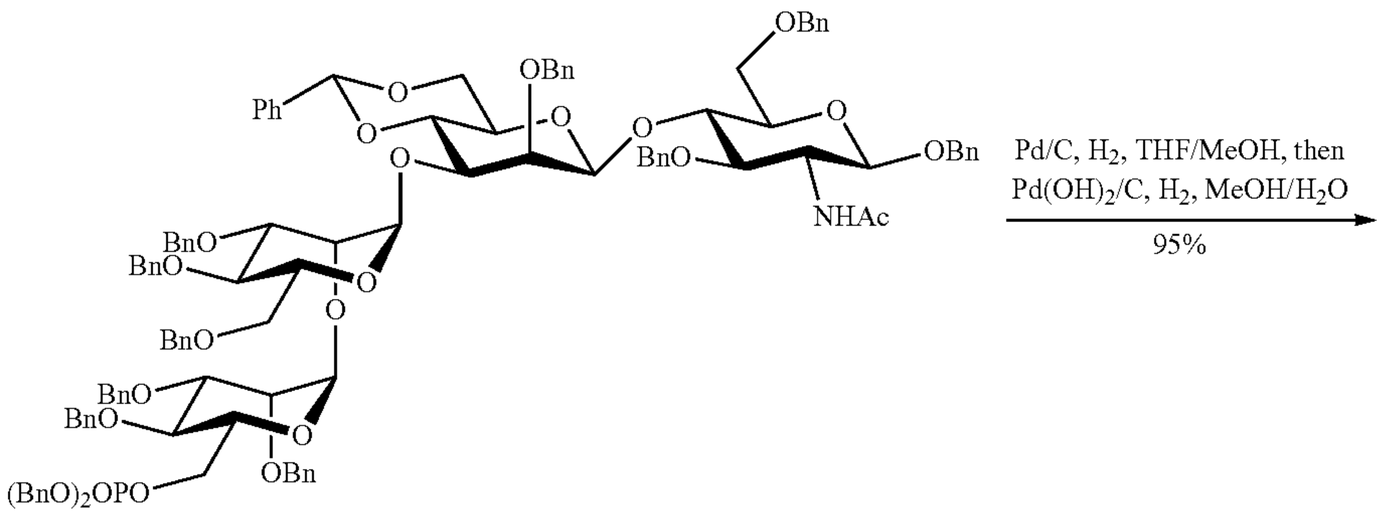


42

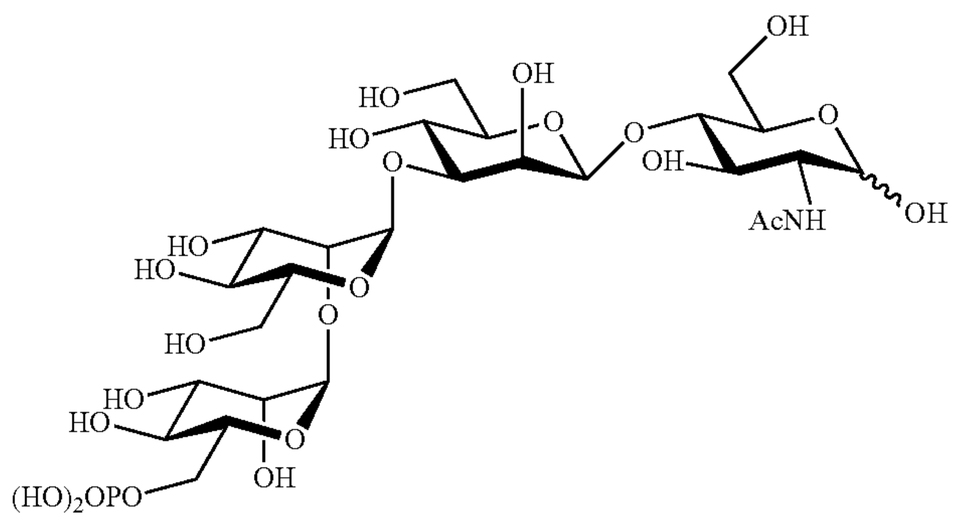
[0226] To a solution of compound 41 (73.6 mg, 0.043 mmol) in anhydrous CH_2Cl_2 (2.5 mL) was added activated 4 Å molecular sieves (250 mg) and tetrazole (0.45 M in MeCN, 482 μL) and the mixture was stirred at room temperature for 1.5 h before $(BnO)_2PNiPr_2$ (58.6 μL) was added. The resulting mixture was further stirred overnight under argon atmosphere at room temperature until the complete disappearance of the starting material. Then the reaction was cooled to −30° C., and mCPBA (77 wt %, 51.5 mg) was added, the reaction mixture was stirred at this temperature for 1 h and then filtered through a Celite pad. The filtrate was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (aq.), dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc=4:1~2:3) to give compound 42 (67.1 mg, 79%) as colorless syrup. R_f=0.20 (hexanes/EtOAc=1:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.41-7.17 (64H, m), 7.06 (1H, t, J=7.5 Hz), 5.91 (1H, d, J=8.2 Hz), 5.44 (1H, s), 5.27 (1H, m), 5.20 (1H, m), 5.06 (1H, dd, J=6.9 Hz, J=11.8 Hz), 5.00-4.79 (10H, m), 4.64-4.43 (14H, m), 4.41-4.36 (2H, m), 4.23 (1H, m), 4.05-3.97 (5H, m), 3.94-3.82 (5H, m), 3.80-3.73 (5H, m), 3.72-3.65 (5H, m), 3.58-3.51 (2H, m), 3.13 (1H, m), 1.72 (3H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 170.15, 138.73, 138.55, 138.39, 138.37, 138.18, 138.13, 138.02, 137.86, 137.64, 137.33, 136.16, 136.08, 136.05, 135.97, 128.55, 128.53, 128.49, 128.44, 128.40, 128.37, 128.32, 128.28, 128.21, 128.18, 128.11, 127.94, 127.93, 127.85, 127.83, 127.79, 127.70, 127.66, 127.58, 127.52, 127.48, 126.11, 101.79, 101.34, 99.62, 99.26, 98.68, 79.95, 79.53, 78.93, 78.77, 77.26, 75.94, 75.44, 75.19, 74.96, 74.71, 74.65, 74.53, 73.91, 73.42, 73.25, 73.09, 72.85, 72.46, 72.06, 71.96, 71.30, 71.23, 70.62, 69.49, 69.15, 69.09, 69.02, 68.97, 68.54, 66.78, 53.94, 29.72, 23.20; 31P NMR (146 MHZ, $CDCl_3$) δ −1.39; MALDI-TOF: $[M+Na]^+$ calcd for $C_{117}H_{122}NNaO_{24}PT$, 1980.21; found, 1979.89.

6-O-phosphonato-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-αβ-D-glucopyranoside (43)

[0227]



42

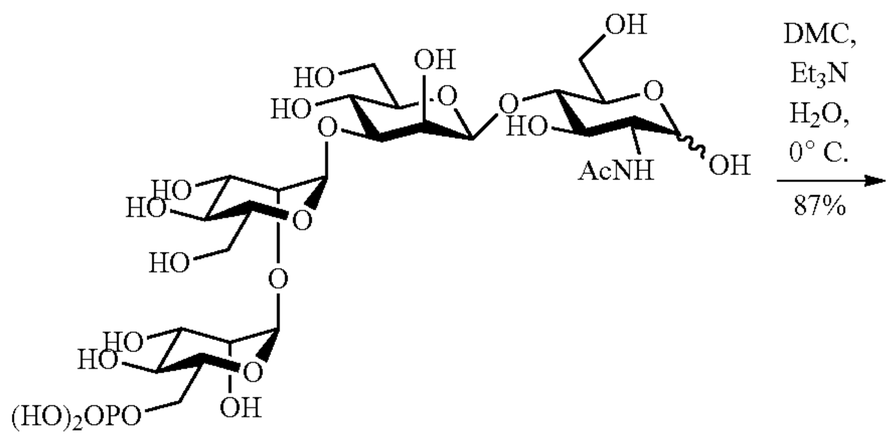


43

[0228] A mixture of compound 42 (67.1 mg, 0.034 mmol) and Pd/C (10 wt. % loading, 40 mg) in MeOH (2.5 mL) and THF (2.5 mL) was stirred under H_2 atmosphere for 21 h. The reaction mixture was filtered through a Celite pad, then concentrated to dryness. The mixture of the residue and $Pd(OH)_2/C$ (20 wt. % loading, 50 mg) in MeOH (4.0 mL) and H_2O (4.0 mL) was stirred under H_2 atmosphere for further 21 h. The reaction mixture was filtered through a Celite pad. The filtrate was concentrated to dryness then dissolved in H_2O and lyophilized. The crude product was purified on a Sephadex G-10 column by elution with H_2O. Fractions containing the product were pooled and lyophilized to give compound 43 (25.6 mg, 95%) as white solid. R_f=0.20 (n-BuOH/EtOH/H_2O/AcOH=1:1:1:0.05); ^{1}H NMR (400 MHZ, D_2O) δ 5.32 (0.96H, s), 5.11 (0.68H, d, J=2.7 Hz), 4.95 (1.17H, s), 4.63-4.61 (0.80H, m), 4.12-4.11 (1.32H, m), 4.01-3.98 (2.47H, m), 3.93-3.90 (2.46H, m), 3.89-3.73 (8.07H, m), 3.73-3.51 (10.71H, m), 3.51-3.38 (1.67H, m), 1.95 (3.00H, s); ^{13}C NMR (100 MHZ, D_2O) δ 174.43, 102.39, 100.66, 99.81, 99.74, 94.92, 90.51, 79.89, 79.22, 78.82, 78.50, 76.10, 74.67, 73.40, 72.54, 72.24, 70.43, 70.39, 70.13, 70.00, 69.94, 69.09, 66.96, 66.47, 66.23, 63.43, 61.05, 60.82, 60.14, 60.00, 59.39, 56.13, 53.66, 22.18, 21.88; 31P NMR (146 MHz, D_2O) δ 4.52 (overlapped signals); HRMS: $[M+H]^+$ calcd for $C_{26}H_{47}NO_{24}P^+$, 788.2220; found, 788.2224.

2-Methyl-[6-0)-phosphonato-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline (8)

[0229]



43

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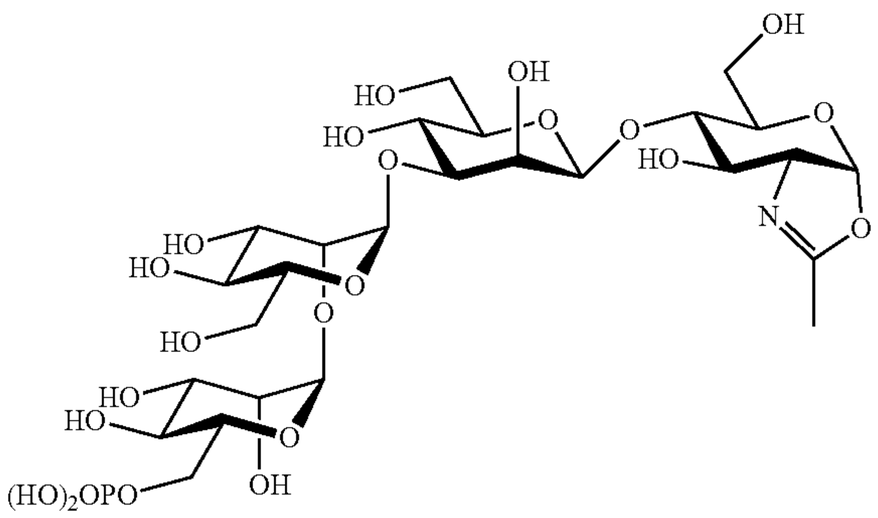
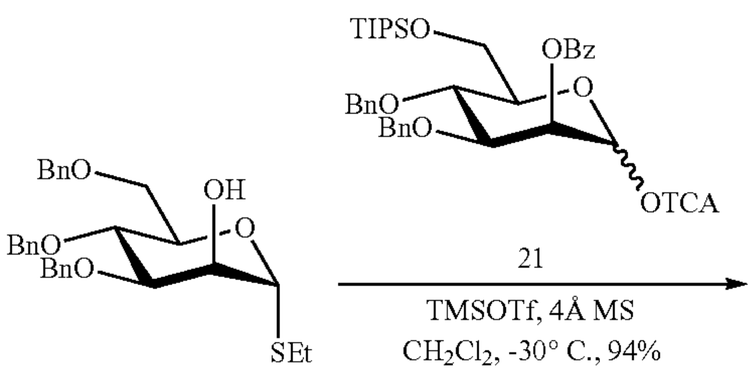


[0230] To a solution of compound 43 (5.0 mg, 0.0064 mmol) in H_2O (250 μL) were added Et_3N (35.6 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 21.5 mg) at 0° C. The reaction mixture was monitored by DIONEX HPAEC-PAD. After 2 h, the HPAEC analysis indicated that the free oligosaccharide was converted into a new oligosaccharide that was eluted earlier than the reducing sugar under the HPAEC condition (see general method). The product was purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound 8 (4.3 mg, 87%) as white solid after lyophilization with 5 mol. % of NaOH. 1H NMR (400 MHZ, D_2O) δ 6.02 (1H, m), 5.35 (1H, m), 4.97 (1H, m), 4.31 (1H, m), 4.05-4.03 (2H, m), 4.01-3.99 (1H, m), 3.96-3.89 (4H, m), 3.83-3.76 (3H, m), 3.75-3.64 (9H, m), 3.62-3.54 (3H, m), 3.46-3.41 (1H, m), 3.37-3.33 (1H, m), 2.00 (3H, s); ^{13}C NMR (100 MHz, D_2O) δ 167.21, 102.43, 100.92, 100.58, 99.87, 79.58, 78.42, 77.33, 76.18, 73.35, 72.77, 72.69, 71.07, 70.37, 70.13, 70.01, 69.13, 66.96, 66.50, 66.45, 62.83, 61.69, 61.02, 60.98, 12.96; 31P NMR (146 MHZ, D_2O) δ 4.52; HRMS: $[M+H]^+$ calcd for $C_{26}H_{45}NO_{23}P^+$, 770.2114; found, 770.2124.

Ethyl 3,4-di-O-benzyl-2-O-benzoyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-1-thio-α-D-mannopyranoside (45)

[0231]



44

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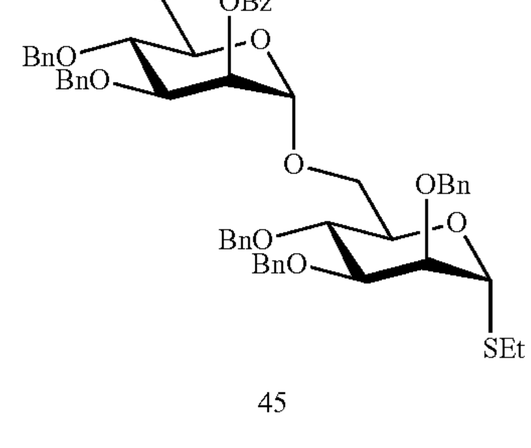
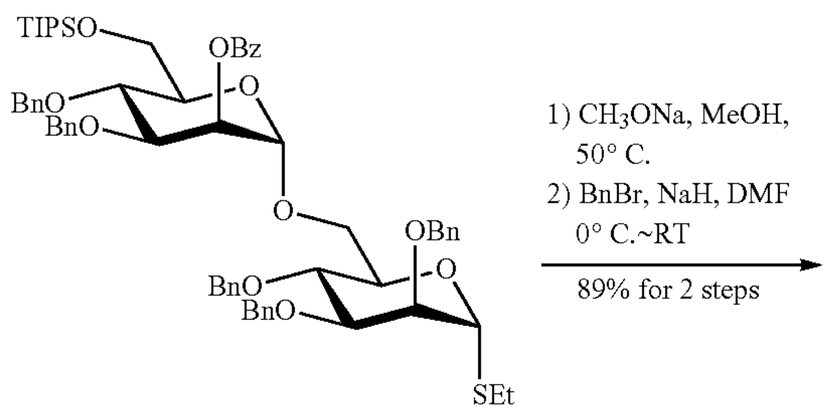


45

[0232] A mixture of trichloroacetimidate donor 21[4] (502 mg, 0.658 mmol), acceptor 44[8] (250 mg, 0.506 mmol) and activated 4 Å molecular sieves (600 mg) in anhydrous CH_2Cl_2 (6.0 mL) was stirred at room temperature under argon atmosphere for 1.5 h, and then cooled to −30° C. TMSOTf (9.25 μL, 0.051 mmol) was added. After stirring at −30° C. for 50 min, the mixture was quenched with triethylamine (50 μL) and filtered then concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes/EtOAc=20:1~10:1) to give product 45 (522 mg, 94%) as white foam. R_f=0.30 (hexanes/EtOAc=10:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.16-8.14, 7.62-7.58, 7.48-7.43, 7.38-7.20 (30H, m, Ar—H), 5.75 (1H, m), 5.39 (1H, m), 5.02-4.92 (3H, m), 4.80-4.75 (2H, m), 4.72-4.66 (2H, m), 4.64-4.58 (2H, m), 4.56-4.51 (2H, m), 4.17-4.06 (3H, m), 4.00-3.92 (3H, m), 3.92-3.86 (3H, m), 3.72-3.70 (2H, m), 2.65-2.51 (2H, m), 1.25 (3H, t, J=7.4 Hz), 1.17-1.08 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.63, 138.98, 138.54, 138.22, 138.14, 138.11, 133.03, 130.17, 130.05, 128.41, 128.31, 128.25, 128.21, 128.19, 128.09, 127.99, 127.87, 127.81, 127.74, 127.69, 127.63, 127.51, 127.40, 98.04, 81.60, 80.52, 78.09, 76.35, 75.10, 74.99, 74.92, 73.96, 72.68, 72.08, 71.98, 71.42, 71.28, 69.01, 66.45, 62.47, 25.29, 18.09, 18.05, 15.04, 12.06; MALDI-TOF: $[M+Na]^+$ calcd for $C_{65}H_{80}NaO_{11}SSi^+$, 1119.51; found, 1119.17.

Ethyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-1-thio-α-D-mannopyranoside (46)

[0233]



45

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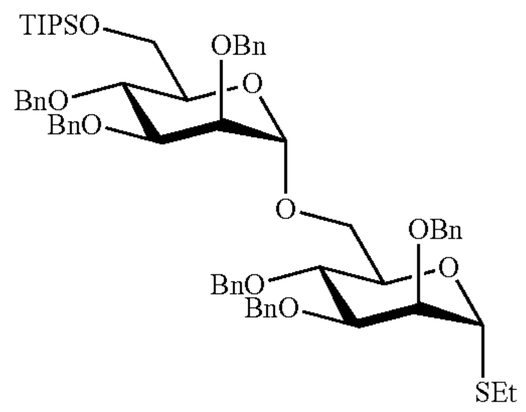
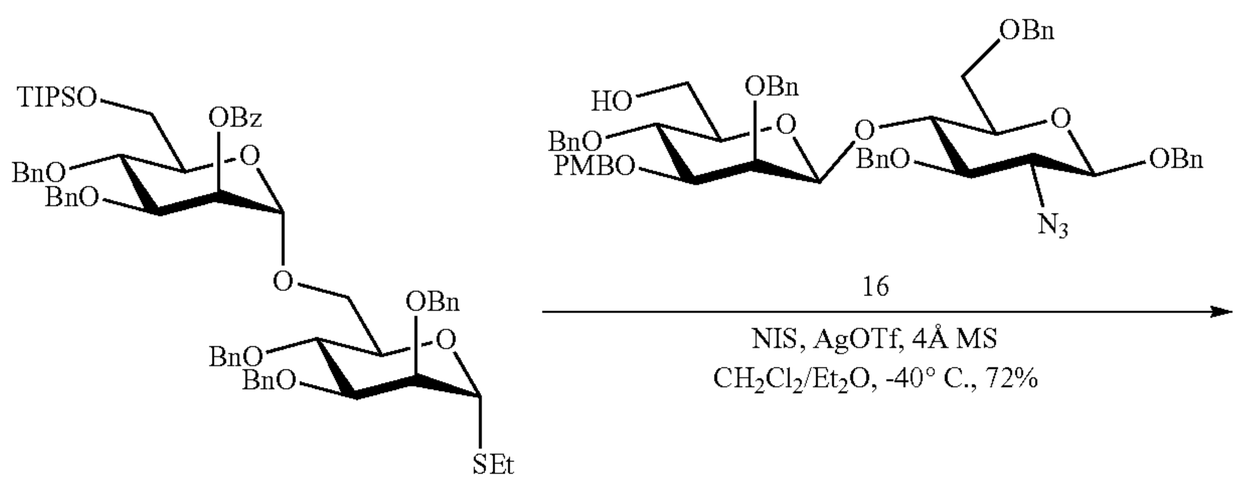


46

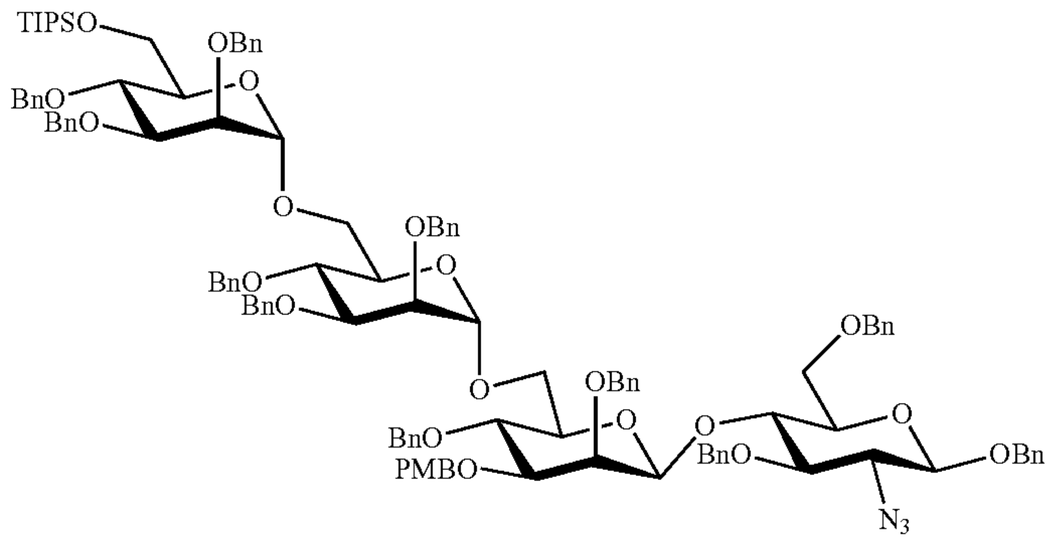
[0234] To a solution of compound 45 (300 mg, 0.274 mmol) in MeOH (4.0 mL) was added sodium methoxide until pH=10, the solution was heated to 50° C. and stirred overnight. After the complete disappearance of the starting material, the solution was concentrated to dryness and dissolved in dry N,N-dimethylformamide (3.0 mL) and cooled to 0° C., sodium hydride (27.4 mg) and benzyl bromide (63.5 μL) were added successively, and the mixture was slowly warmed to room temperature. After the completion of the reaction as monitored by TLC, MeOH was added to quench the excess sodium hydride. The reaction was diluted with CH_2Cl_2, successively washed with H_2O and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash column chromatography (hexanes/EtOAc=20:1~10:1) to afford compound 46 (265 mg, 89% for 2 steps) as colorless syrup. R_f=0.60 (hexanes/EtOAc=10:1); 1H NMR (400 MHZ, $CDCl_3$) δ 7.40-7.23 (30H, m, Ar—H), 5.35 (1H, m), 5.05 (1H, m), 4.97-4.91 (2H, m), 4.73-4.52 (10H, m), 4.10-4.05 (1H, m), 4.04-3.98 (1H, m), 3.95-3.85 (8H, m), 3.71-3.63 (2H, m), 2.63-2.48 (2H, m), 1.23 (3H, dt, J=7.3 Hz, J=0.85 Hz), 1.10-1.07 (21H, m); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 139.06, 138.83, 138.58, 138.54, 138.24, 138.08, 128.40, 128.34, 128.31, 128.23, 128.16, 127.91, 127.87, 127.84, 127.78, 127.75, 127.69, 127.54, 127.47, 127.38, 127.28, 97.76, 81.80, 80.50, 79.73, 76.50, 75.27, 75.06, 74.97, 74.93, 74.70, 73.34, 72.34, 72.20, 72.07, 71.93, 71.66, 65.82, 63.01, 25.27, 18.06, 18.02, 15.05, 12.06; MALDI-TOF: $[M+Na]^+$ calcd for $C_{65}H_{82}NaO_{10}SSi^+$, 1105.53; found, 1105.15.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (47)

[0235]



46

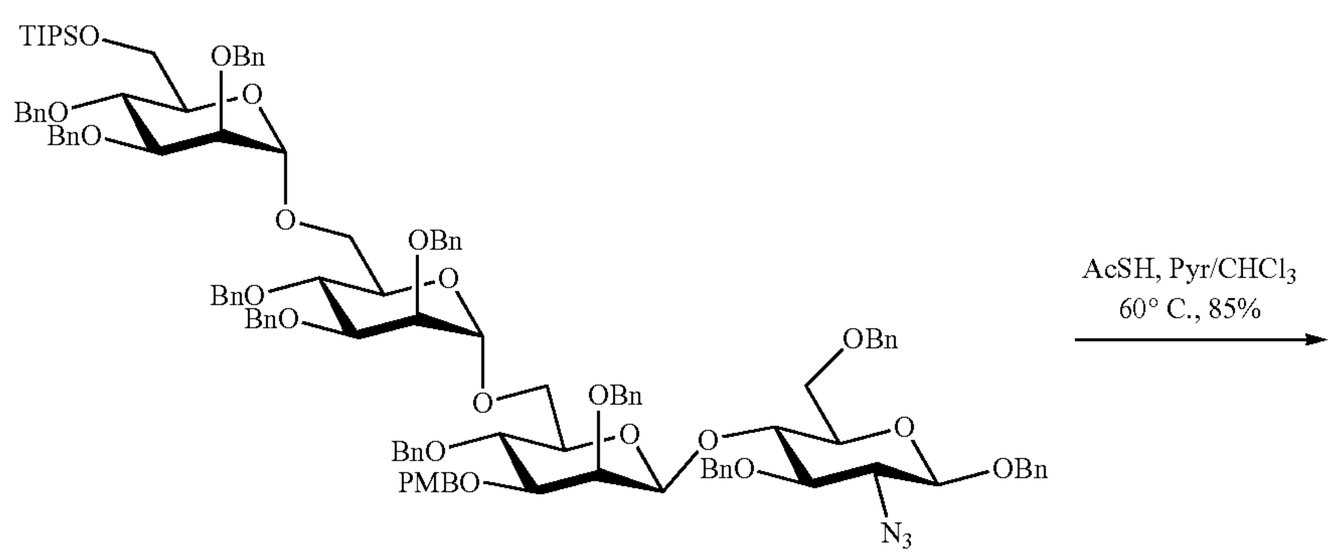


47

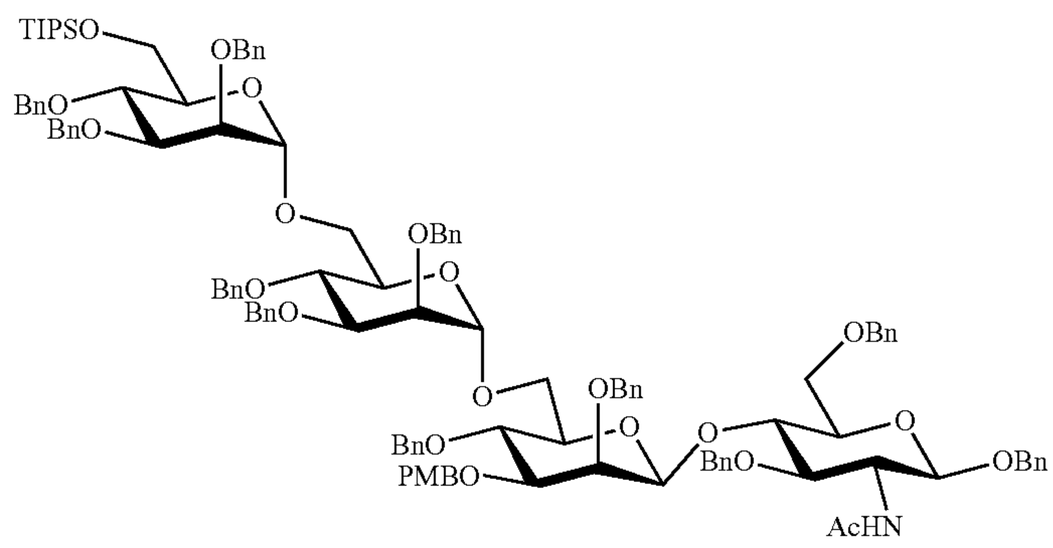
[0236] A mixture of compound 46 (120 mg, 0.111 mmol), acceptor 16 (80 mg, 0.085 mmol) and activated 4 Å molecular sieves (400 mg) in anhydrous CH_2Cl_2/Et_{20} (3 mL/1 mL) was stirred at room temperature under argon atmosphere for 1.5 h, and then cooled to −40° C. N-iodosuccinimide (38.2 mg, 0.170 mmol) and AgOTf (4.4 mg, 0.017 mmol) were successively added. After stirring at −40° C. for 1 h, the mixture was quenched with triethylamine (10 μL) and filtered then concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes/EtOAc=10:1~4:1) to give the desired product 47 (120 mg, 72%) as colorless oil along with ß isomer (29.5 mg, 17%). R_f=0.40 (hexanes/EtOAc=4:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.41-7.17 (57H, m, Ar—H), 6.87-6.85 (2H, m, Ar—H), 5.04-4.98 (2H, m), 4.94-4.76 (8H, m), 4.70-4.40 (15H, m), 4.37-4.28 (3H, m), 4.07-4.02 (1H, m), 4.00-3.64 (16H, m), 3.57-3.42 (4H, m), 3.41-3.33 (4H, m), 3.29-3.26 (1H, m), 1.14-1.00 (21H, m); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 170.64, 158.80, 138.73, 138.59, 138.40, 138.35, 138.16, 138.10, 138.06, 137.96, 137.94, 137.49, 136.44, 129.70, 128.75, 128.71, 128.13, 128.04, 127.97, 127.84, 127.79, 127.77, 127.74, 127.67, 127.58, 127.44, 127.41, 127.36, 127.32, 127.27, 127.13, 127.07, 127.00, 126.96, 126.87, 126.82, 126.77, 126.62, 113.36, 101.05, 100.16, 97.87, 97.71, 82.17, 80.61, 79.44, 78.96, 76.94, 74.91, 74.83, 74.68, 74.60, 74.42, 74.29, 73.98, 73.83, 73.78, 73.70, 73.12, 72.67, 72.28, 71.75, 71.43, 71.02, 70.85, 70.43, 68.21, 66.05, 65.55, 65.16, 62.30, 59.91, 54.79, 20.56, 17.59, 17.55, 13.73, 11.59; MALDI-TOF: $[M+Na]^+$ calcd for $C_{118}H_{135}N_3NaO_{21}Si^+$, 1980.93; found, 1981.54.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (48)

[0237]



47

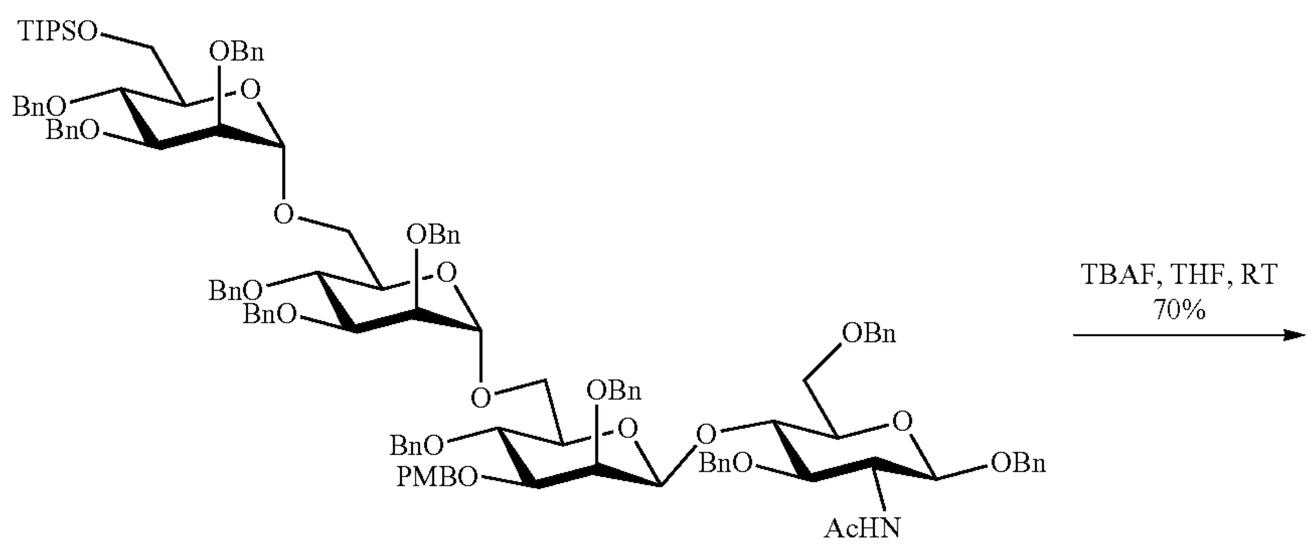


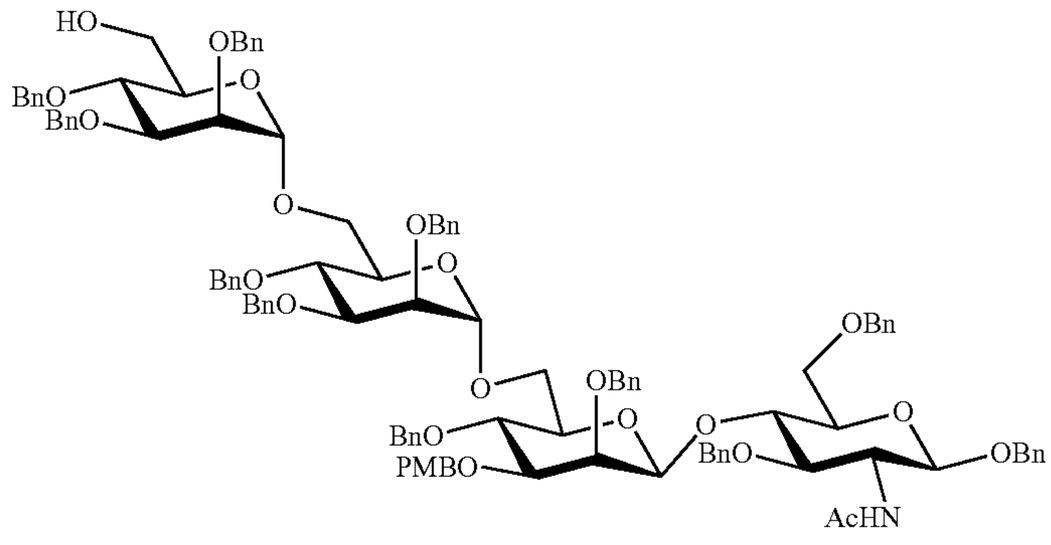
48

[0238] A solution of compound 47 (72.0 mg, 0.037 mmol) in a mixture of AcSH/pyridine/$CHCl_3$ (0.6 mL/0.4 mL/0.6 mL) was stirred at 60° C. for 20 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=6:1~2:1) to afford compound 48 (61.7 mg, 85%) as colorless syrup. R_f=0.30 (hexanes/EtOAc=2:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.44-7.16 (57H, m, Ar—H), 6.88-6.86 (2H, m, Ar—H), 5.42 (1H, d, J=7.6 Hz, NH), 5.01-4.84 (10H, m), 4.67-4.38 (18H, m), 4.10-4.02 (3H, m), 3.96-3.94 (1H, m), 3.89-3.77 (13H, m), 3.67-3.64 (2H, m), 3.57-3.53 (2H, m), 3.48-3.42 (2H, m), 3.36-3.33 (1H, m), 3.27-3.25 (1H, m), 1.51 (3H, s), 1.14-1.06 (21H, m); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 169.87, 158.77, 148.48, 138.66, 138.58, 138.42, 138.14, 138.09, 138.05, 138.02, 137.93, 137.60, 137.36, 136.24, 129.70, 128.74, 128.10, 127.98, 127.84, 127.78, 127.70, 127.66, 127.56, 127.53, 127.40, 127.37, 127.33, 127.29, 127.25, 127.18, 127.15, 127.07, 127.01, 126.96, 126.86, 126.81, 126.61, 123.54, 113.34, 100.52, 98.75, 97.61, 97.57, 82.11, 79.75, 78.96, 77.16, 74.92, 74.65, 74.47, 74.39, 74.35, 74.22, 74.13, 74.04, 73.95, 73.37, 73.07, 72.64, 72.29, 71.70, 71.41, 71.32, 70.96, 70.84, 70.39, 68.82, 66.11, 65.10, 62.31, 54.79, 22.63, 17.59, 17.55, 11.59; MALDI-TOF: $[M+Na]^+$ calcd for $C_{120}H_{139}NNaO_{22}Si^+$, 1996.95; found, 1997.36.

Benzyl 2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (49)

[0239]

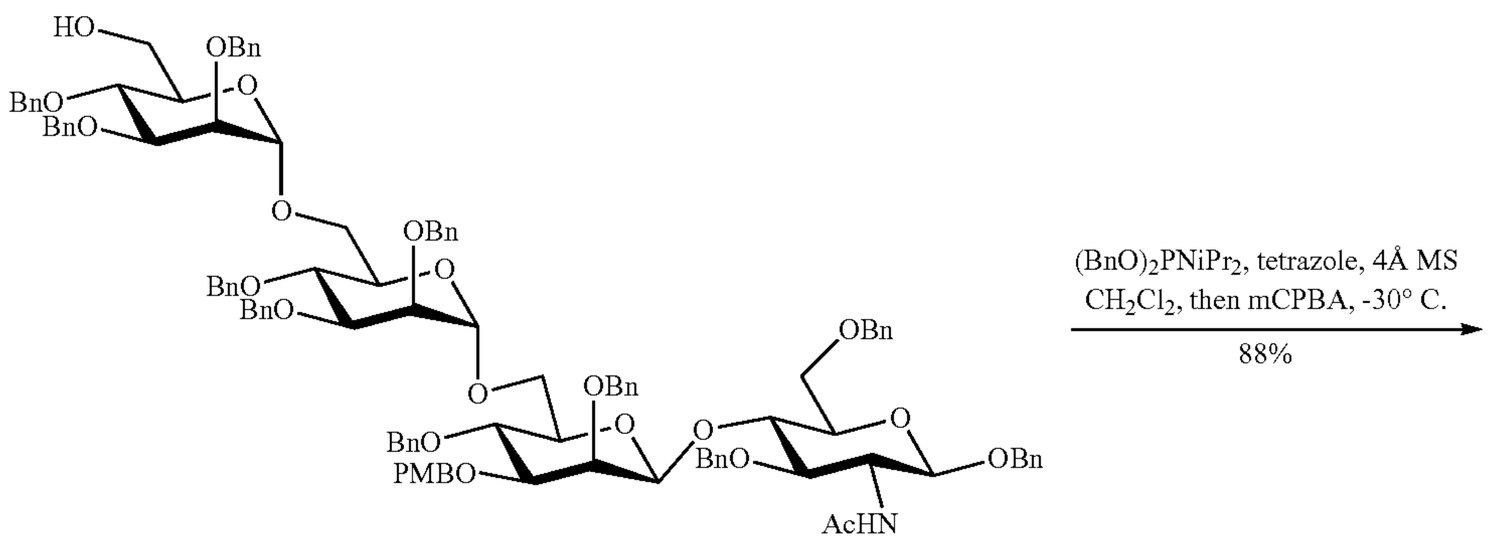


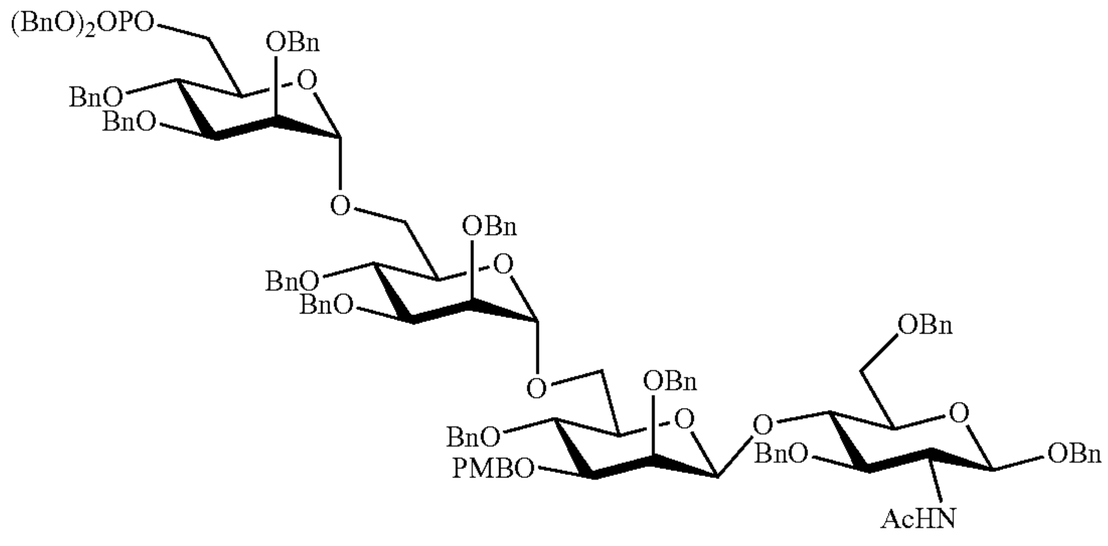


[0240] To a solution of compound 48 (61.7 mg, 0.031 mmol) in THF (1.2 mL) was added TBAF (1 M in THF, 174 μL), and the mixture was stirred at room temperature for 20 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=6:1~1:1) to afford compound 49 (40.0 mg, 70%) as colorless syrup. R_f=0.30 (hexanes/EtOAc=1:1); 1H NMR (400 MHZ, $CDCl_3$) δ 7.43-7.41, 7.35-7.20 (57H, m, Ar—H), 6.89-6.87 (2H, m, Ar—H), 5.46 (1H, d, J=7.5 Hz, NH), 5.10-5.06 (2H, m), 4.99-4.94 (4H, m), 4.92-4.85 (4H, m), 4.69-4.61 (5H, m), 4.59-4.53 (6H, m), 4.50-4.38 (7H, m), 4.17 (1H, dd, J=8.4 Hz, J=8.4 Hz), 4.05 (1H, dd, J=8.2 Hz, J=8.2 Hz), 4.00-3.94 (2H, m), 3.92-3.76 (12H, m), 3.75-3.69 (3H, m), 3.67-3.62 (3H, m), 3.59-3.55 (1H, m), 3.48-3.45 (1H, m), 3.41-3.32 (2H, m), 3.25-3.22 (1H, m), 1.53 (3H, s); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 170.29, 159.26, 139.25, 138.81, 138.67, 138.65, 138.59, 138.53, 138.45, 138.43, 138.28, 138.12, 137.89, 130.20, 129.23, 128.51, 128.45, 128.39, 128.34, 128.32, 128.30, 128.26, 128.20, 128.15, 128.05, 128.02, 127.96, 127.90, 127.84, 127.75, 127.74, 127.72, 127.69, 127.64, 127.58, 127.55, 127.51, 127.49, 127.46, 127.43, 127.37, 113.84, 100.81, 99.22, 97.90, 82.57, 80.31, 79.22, 77.53, 75.34, 75.30, 75.16, 75.12, 75.05, 74.99, 74.93, 74.79, 74.76, 74.57, 74.15, 73.60, 72.80, 72.45, 72.15, 71.92, 71.76, 71.41, 71.36, 71.05, 69.31, 66.75, 65.31, 62.31, 56.54, 55.31, 29.74, 23.25; MALDI-TOF: $[M+Na]^+$ calcd for $C_{11}H_{119}NNaO_{22}^+$, 1840.81; found, 1841.28.

Benzyl 2,3,4-tri-O-benzyl-6-O-dibenzylphospho-nato-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-ben-zyl-α-D-mannopyranosyl-(1→6)-2,4-di-O-benzyl-3-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (50)

[0241]

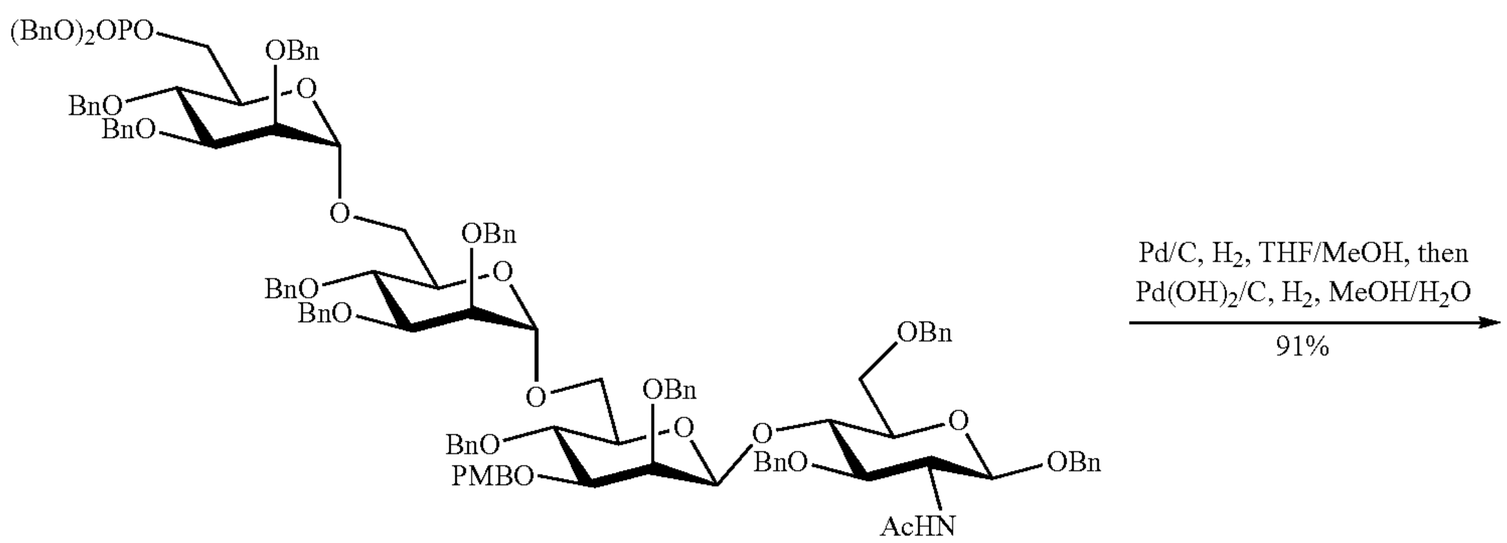


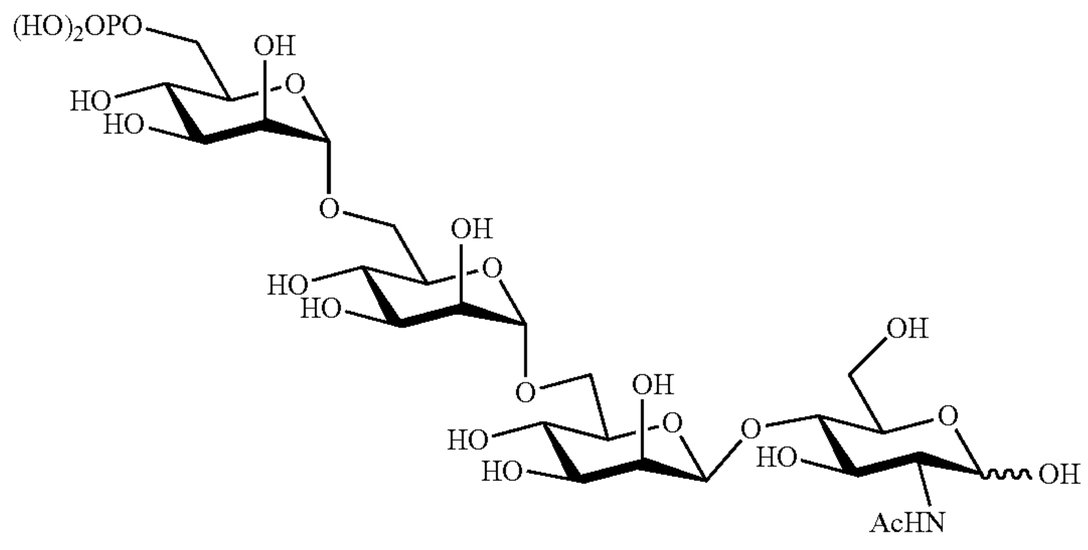


[0242] To a solution of compound 49 (40.0 mg, 0.022 mmol) in anhydrous CH_2Cl_2 (1.5 mL) was added activated 4 Å molecular sieves (150 mg) and tetrazole (0.45 M in MeCN, 244 μL) and the mixture was stirred at room temperature for 1.5 h before $(BnO)_2PNiPr_2$ (37.3 μL) was added. The resulting mixture was further stirred overnight under argon atmosphere at room temperature until the complete disappearance of the starting material. Then the reaction was cooled to −30° C., and mCPBA (77 wt %, 26.1 mg) was added, the reaction mixture was stirred at this temperature for 1 h and then filtered through a Celite pad. The filtrate was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (aq.), dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc=6:1~3:2) to give compound 50 (40.0 mg, 88%) as colorless syrup. R_f=0.15 (hexanes/EtOAc=3:2); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.43-7.18 (67H, m, Ar—H), 6.89-6.87 (2H, m, Ar—H), 5.45 (1H, d, J=7.6 Hz, NH), 5.13-5.01 (6H, m), 4.99-4.83 (8H, m), 4.67-4.34 (18H, m), 4.26-4.24 (2H, m), 4.18-4.11 (1H, m), 4.06-4.01 (2H, m), 3.97-3.92 (1H, m), 3.89-3.77 (12H, m), 3.69-3.63 (3H, m), 3.60-3.55 (2H, m), 3.49-3.46 (1H, m), 3.40-3.34 (2H, m), 3.26-3.23 (1H, m), 1.53 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.35, 159.27, 139.25, 138.84, 138.64, 138.61, 138.57, 138.49, 138.45, 138.21, 138.12, 137.91, 130.20, 129.25, 128.49, 128.47, 128.38, 128.34, 128.29, 128.21, 128.17, 128.06, 128.00, 127.91, 127.86, 127.83, 127.80, 127.75, 127.67, 127.51, 127.44, 127.37, 113.84, 100.95, 99.27, 98.00, 97.93, 82.60, 80.22, 79.10, 77.79, 75.31, 75.23, 75.09, 75.00, 74.92, 74.79, 74.55, 74.07, 73.83, 73.58, 72.81, 72.38, 71.89, 71.43, 71.17, 70.96, 69.34, 69.29, 69.19, 69.13, 66.52, 65.56, 56.36, 55.31, 29.74, 23.24; 31P NMR (146 MHZ, $CDCl_3$) δ −1.13; MALDI-TOF: $[M+Na]^+$ calcd for $C_{125}H_{132}NNaO_{25}P^+$, 2100.87; found, 2101.36.

6-O-phosphonato-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-αβ-D-glucopyranoside (51)

[0243]

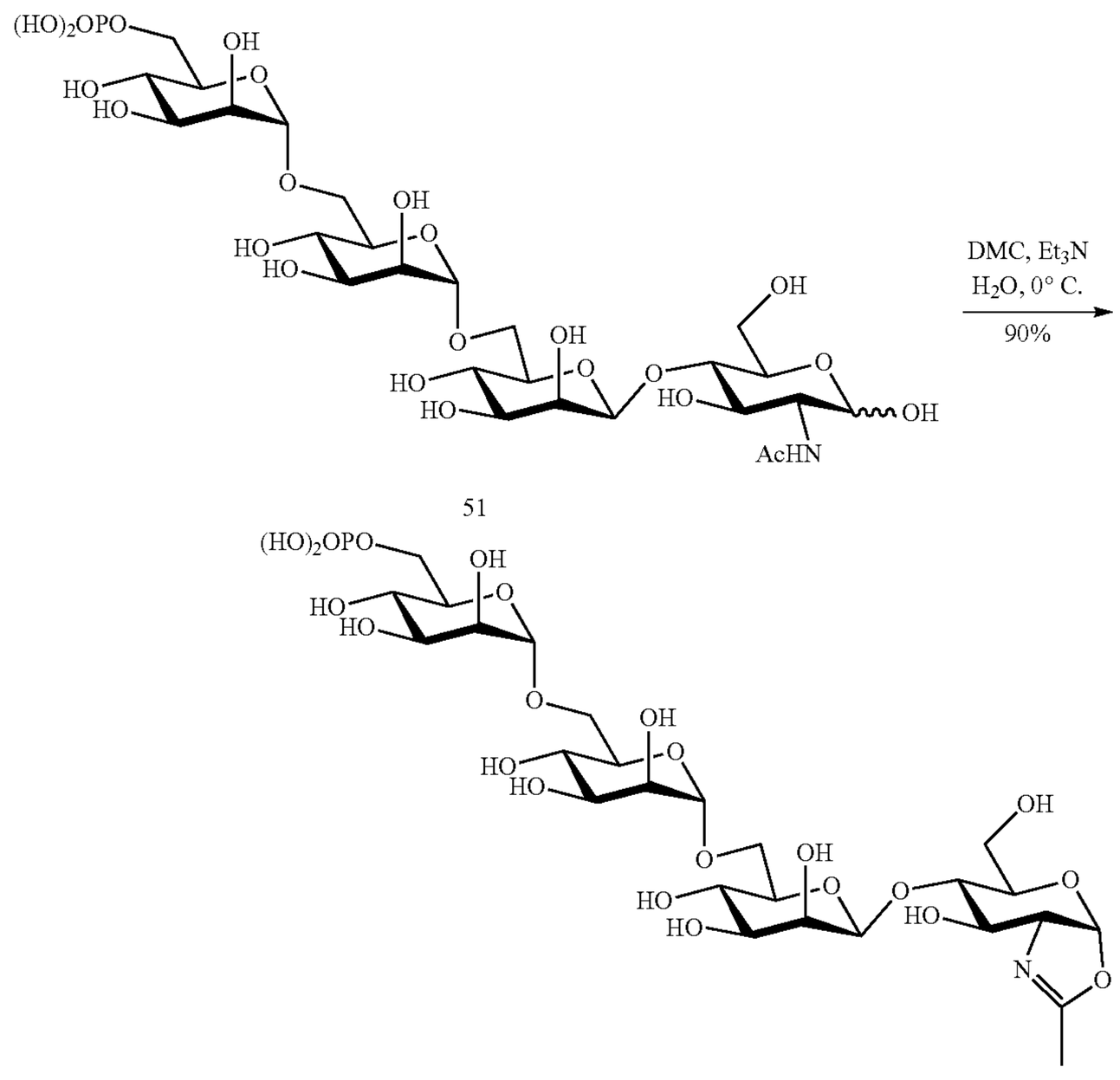




[0244] A mixture of compound 50 (40.0 mg, 0.019 mmol) and Pd/C (10 wt. % loading, 20 mg) in MeOH (1.5 mL) and THF (1.5 mL) was stirred under H_2 atmosphere for 21 h. The reaction mixture was filtered through a Celite pad, then concentrated to dryness. The mixture of the residue and $Pd(OH)_2/C$ (20 wt. % loading, 30 mg) in MeOH (2.0 mL) and H_2O (2.0 mL) was stirred under H_2 atmosphere for further 22 h. The reaction mixture was filtered through a Celite pad. The filtrate was concentrated to dryness then dissolved in H_2O and lyophilized. The crude product was purified on a Sephadex G-10 column by elution with H_2O. Fractions containing the product were pooled and lyophilized to give compound 51 (13.7 mg, 91%) as white solid. R_f=0.20 (n-BuOH/EtOH/H_2O/AcOH=1:1:1:0.05); ^{1}H NMR (400 MHZ, D_2O) δ 5.10 (0.67H, m), 4.80 (3.45H, m), 4.62 (0.54H, m), 4.04-3.96 (3.49H, m), 3.91-3.81 (4.95H, m), 3.81-3.73 (4.83H, m), 3.73-3.64 (6.54H, m), 3.64-3.58 (3.02H, m), 3.58-3.45 (3.36H, m), 1.95 (3.00H, s); ^{13}C NMR (100 MHZ, D_2O) δ 174.45, 174.13, 100.17, 100.07, 99.38, 99.34, 99.02, 98.93, 94.41, 90.03, 79.85, 79.61, 73.95, 73.77, 72.30, 71.89, 71.23, 71.18, 70.32, 70.22, 70.17, 70.12, 70.02, 69.97, 69.48, 69.41, 68.66, 66.30, 66.06, 65.80, 65.76, 65.15, 65.08, 63.55, 59.82, 59.70, 55.54, 53.13, 46.23, 21.83, 21.51; 31P NMR (146 MHz, D_2O) δ 0.73 (overlapped signals); HRMS: $[M+H]^+$ calcd for $C_{26}H_{47}NO_{24}P^+$, 788.2220; found, 788.2228.

2-Methyl-[6-O-phosphonato-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline (9)

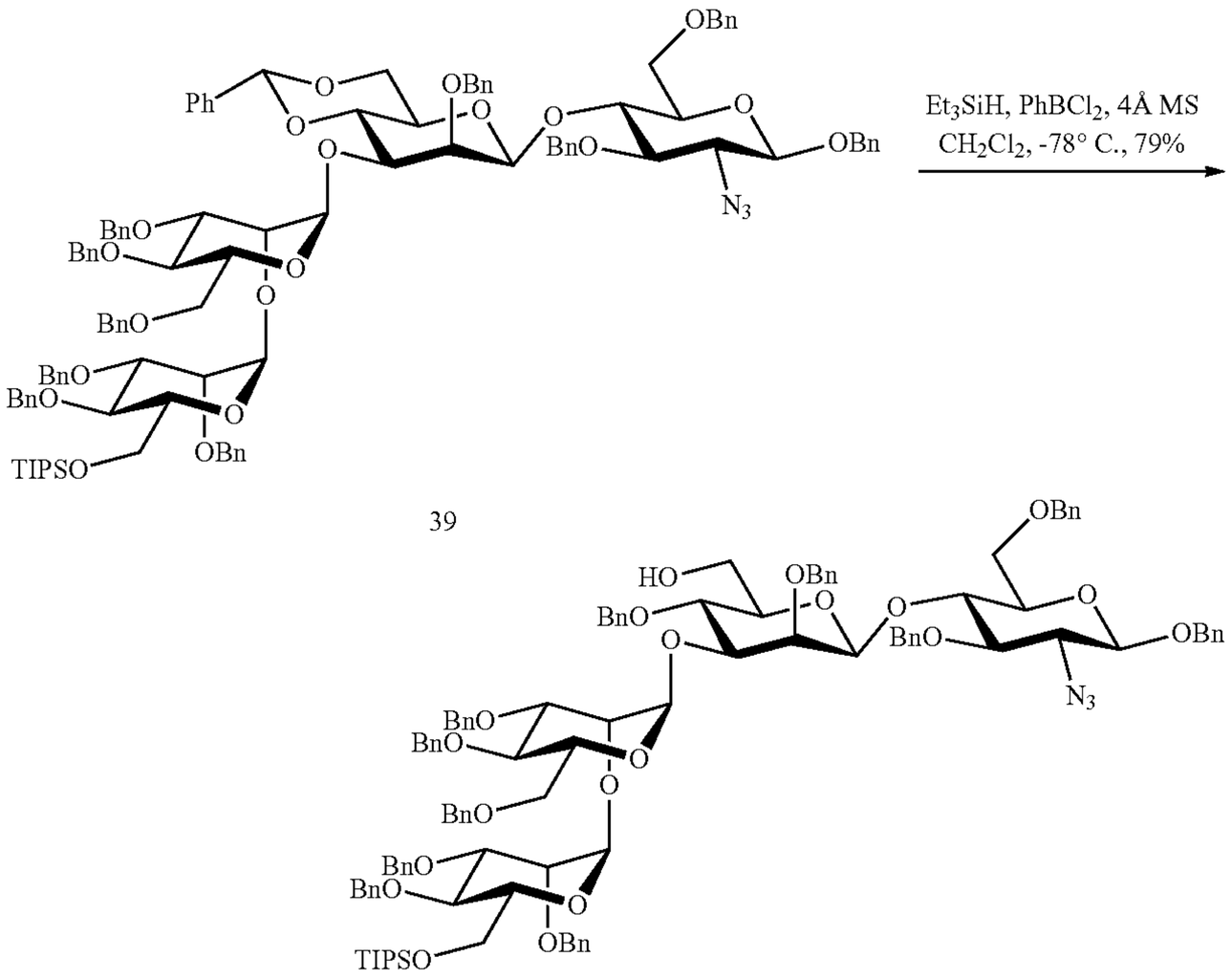
[0245]



[0246] To a solution of compound 51 (7.0 mg, 0.009 mmol) in H_2O (250 μL) were added Et_3N (60 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 30 mg) at 0° C. The reaction mixture was monitored by DIONEX HPAEC-PAD. After 2 h, the HPAEC analysis indicated that the free oligosaccharide was converted into a new oligosaccharide that was eluted earlier than the reducing sugar under the HPAEC condition (see general method). The product was purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound 9 (6.1 mg, 90%) as white solid after lyophilization with 5 mol. % of NaOH. 1H NMR (400 MHZ, D_2O) δ 6.02 (1H, d, J=7.3 Hz), 4.88-4.83 (3H, m), 4.65 (1H, m), 4.31-4.29 (1H, m), 4.14-4.11 (1H, m), 4.01-3.87 (8H, m), 3.86-3.84 (1H, m), 3.80-3.76 (4H, m), 3.75-3.65 (9H, m), 3.60-3.53 (4H, m), 3.49-3.47 (1H, m), 3.37-3.34 (1H, m), 1.99 (3H, d, J=1.6 Hz); ^{13}C NMR (100 MHz, D_2O) δ 168.25, 101.49, 99.91, 99.71, 99.67, 77.69, 74.43, 72.90, 72.23, 72.16, 70.89, 70.79, 70.48, 70.26, 70.05, 69.86, 69.18, 69.10, 66.73, 66.58, 66.42, 66.25, 66.10, 65.86, 65.62, 65.13, 62.76, 61.72, 12.97; 31P NMR (146 MHz, D_2O) δ 3.99; HRMS: $[M+H]^+$ calcd for $C_{26}H_{45}NO_{23}P^+$, 770.2114; found, 770.2133.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-0)-benzyl-2-deoxy-β-D-glucopyranoside (52)

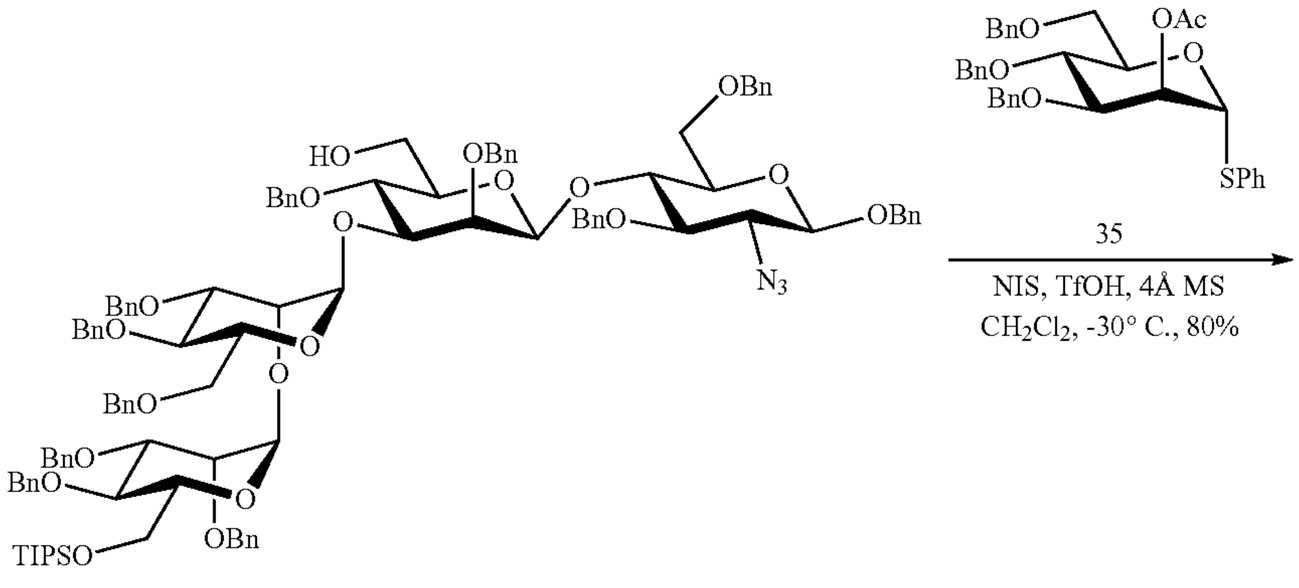
[0247]



[0248] A mixture of compound 39 (130 mg, 0.071 mmol) and activated 4 Å molecular sieves (250 mg) in anhydrous CH_2Cl_2 (2.5 mL) was stirred for 1.5 h at room temperature then cooled to −78° C. Et_3SiH (89.6 μL, 0.565 mmol) and $PhBCl_2$ (45.6 μL, 0.353 mmol) were added. The resulting mixture was stirred for 2.5 h under argon at −78° C., then Et_3N (135 μL) was added to quench the reaction. The residue was filtered through a Celite pad, diluted with CH_2Cl_2, washed with saturated $NaHCO_3$(aq) and brine, dried over Na_2SO_4, and concentrated to dryness. Flash chromatography on silica gel (hexane/EtOAc=10:1~ 3:1) gave compound 52 as colorless syrup (103 mg, 79%). R_f=0.30 (hexanes/EtOAc=4:1); Spectroscopic data were in agreement with literature values.[7] MALDI-TOF: $[M+Na]^+$ calcd for $C_{110}H_{127}N_3NaO_{20}Si^+$, 1862.30; found, 1862.04.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (53)

[0249]



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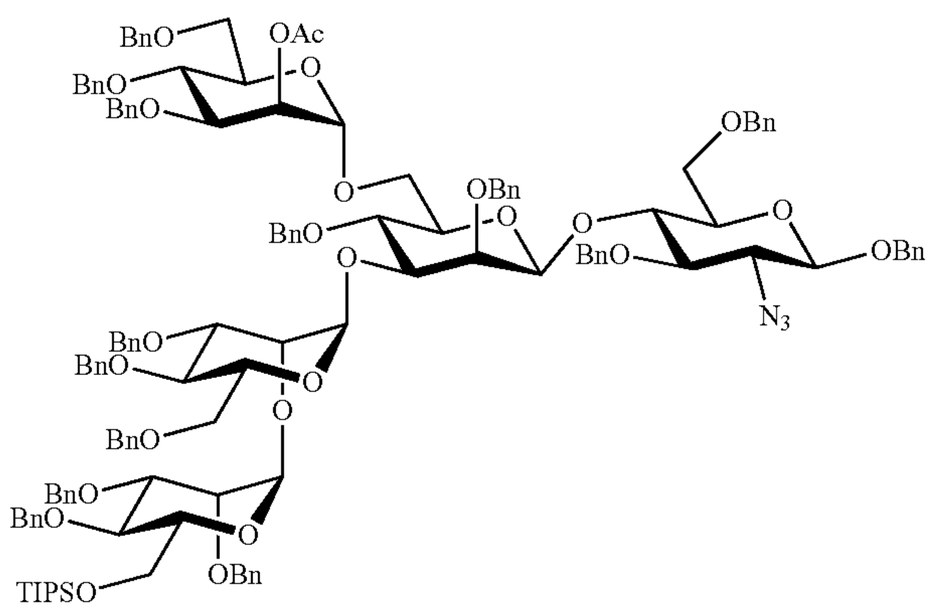
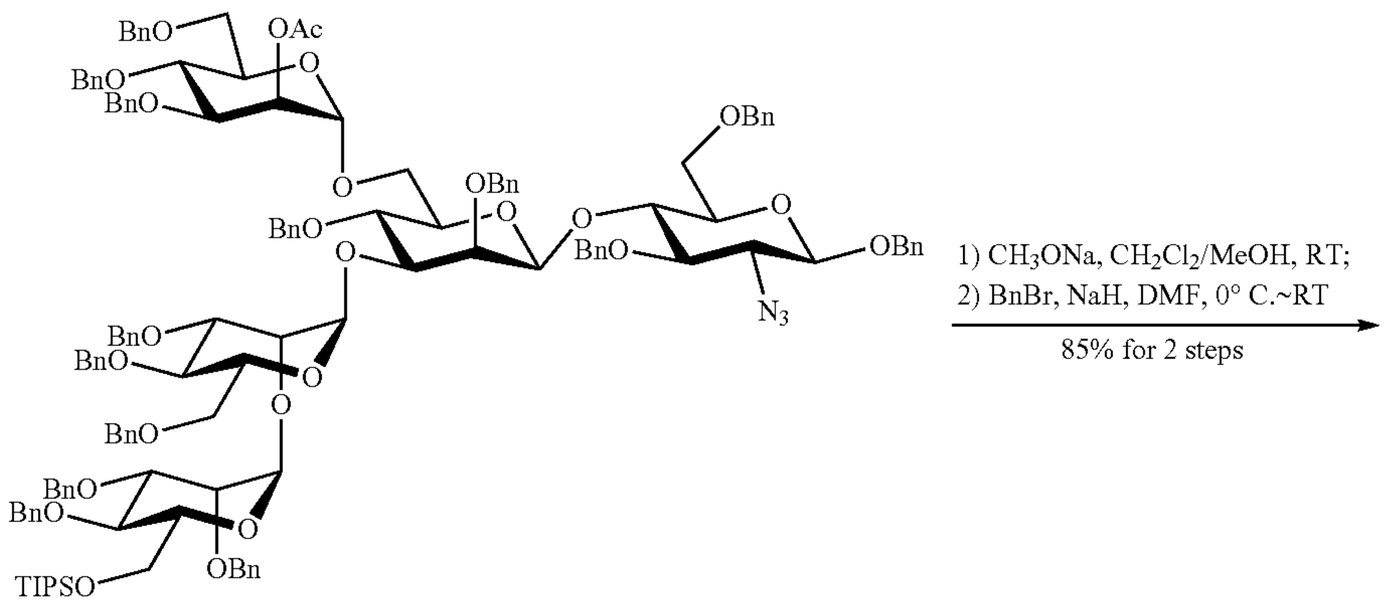


[0250] A mixture of compound 35[6] (20 mg, 0.034 mmol), acceptor 52 (34 mg, 0.018 mmol) and activated 4 Å molecular sieves (100 mg) in anhydrous CH_2Cl_2 (1.0 mL) was stirred at room temperature under argon atmosphere for 1.5 h, and then cooled to −30° C. N-iodosuccinimide (14.7 mg, 0.065 mmol) and TfOH (0.38 μL, 0.004 mmol) were successively added. After stirring at −30° C. for 40 min, the mixture was quenched with triethylamine (5 μL) and filtered then concentrated in vacuo. The residue was purified via silica gel chromatography (hexanes/EtOAc=10:1~4:1) to give the pentasaccharide 53 (34 mg, 80%) as colorless syrup. R_f=0.40 (hexanes/EtOAc=4:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.40-7.17 (70H, m), 5.38 (1H, m), 5.23 (1H, m), 5.13 (1H, m), 5.07 (1H, d, J=11.3 Hz), 5.00 (1H, d, J=11.3 Hz), 4.96-4.92 (2H, m), 4.88 (1H, d, J=5.9 Hz), 4.84 (1H, m), 4.80 (1H, d, J=7.2 Hz), 4.78-4.75 (2H, m), 4.72 (1H, m), 4.70-4.62 (4H, m), 4.60 (1H, m), 4.57-4.52 (6H, m), 4.51-4.40 (7H, m), 4.32-4.30 (1H, m), 4.29-4.21 (3H, m), 4.05-3.91 (7H, m), 3.90-3.84 (3H, m), 3.81-3.75 (2H, m), 3.70-3.54 (11H, m), 3.51-3.45 (2H, m), 3.32 (1H, dd, J=9.1 Hz, J=9.1 Hz), 3.25-3.17 (3H, m), 2.08 (3H, s), 1.06 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.12, 139.07, 138.99, 138.85, 138.74, 138.65, 138.58, 138.54, 138.47, 138.40, 138.06, 138.05, 137.98, 137.95, 137.01, 128.53, 128.43, 128.37, 128.32, 128.29, 128.27, 128.24, 128.21, 128.20, 128.15, 128.07, 128.05, 128.01, 127.91, 127.85, 127.75, 127.71, 127.65, 127.56, 127.51, 127.48, 127.26, 101.51, 101.09, 100.48, 98.56, 97.81, 82.28, 81.14, 80.22, 79.50, 78.71, 78.16, 77.26, 75.16, 75.02, 74.99, 74.87, 74.80, 74.62, 74.52, 74.43, 74.20, 74.05, 73.83, 73.42, 73.35, 73.19, 72.98, 72.44, 72.14, 71.62, 71.41, 70.71, 69.96, 68.56, 68.40, 66.68, 65.70, 62.46, 29.74, 21.07, 18.09, 18.02, 12.06; MALDI-TOF: $[M+Na]^+$ calcd for $C_{139}H_{158}N_3O_{26}Si^+$, 2336.85; found, 2336.24.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (54)

[0251]



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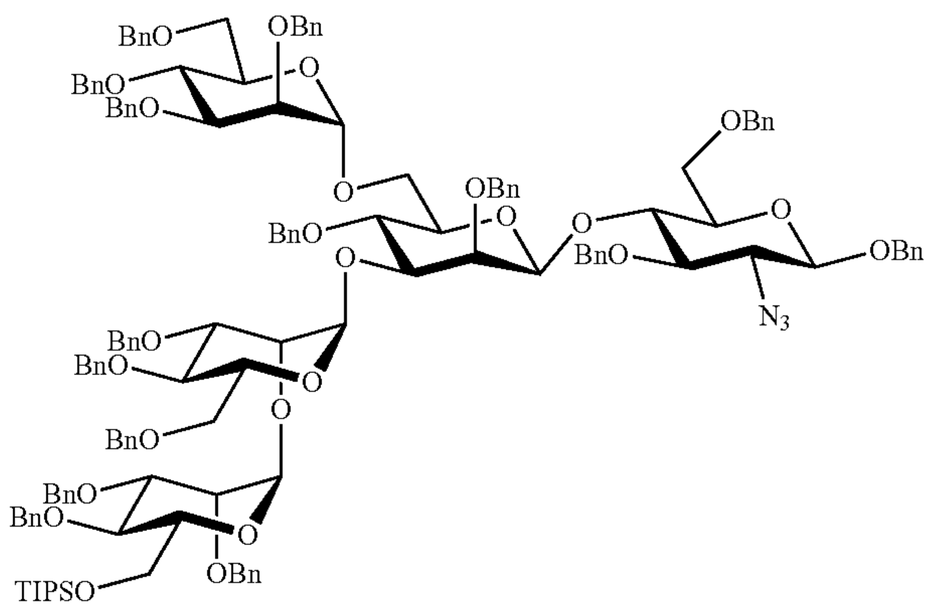
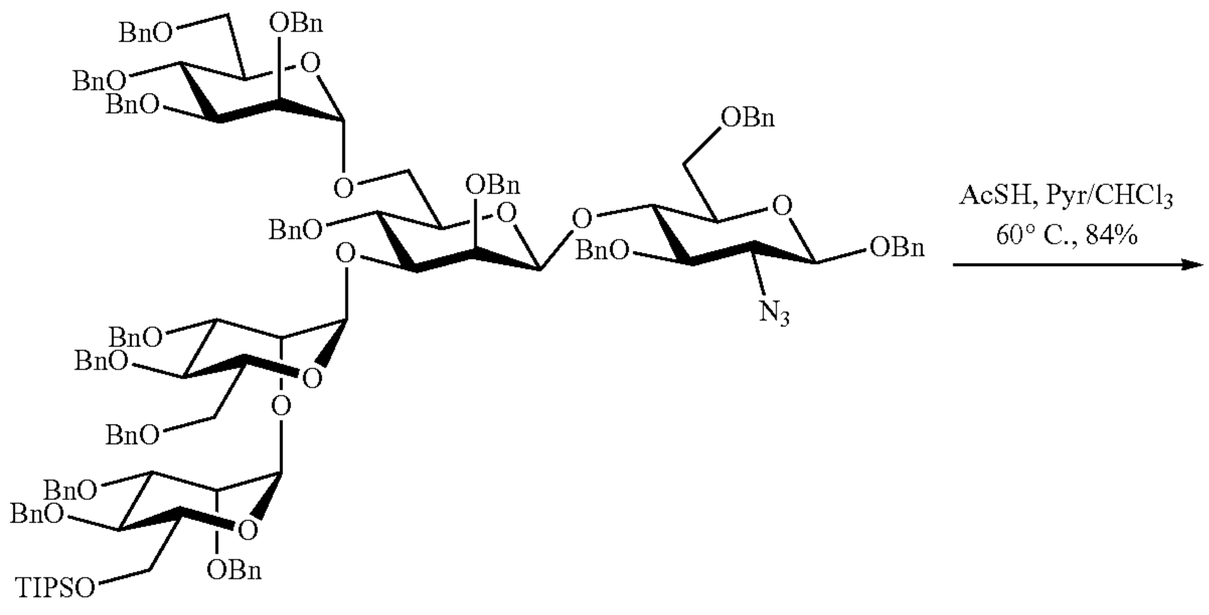


[0252] To a solution of compound 53 (34.0 mg, 0.0147 mmol) in MeOH (1.0 mL) and CH_2Cl_2 (1.0 mL) was added sodium methoxide until pH=10, the solution was stirred at room temperature overnight. After the complete disappearance of the starting material, the solution was concentrated to dryness and dissolved in dry N,N-dimethylformamide (2.0 mL) and cooled to 0° C., sodium hydride (3.6 mg, 0.088 mmol) and benzyl bromide (8.5 μL, 0.074 mmol) were added successively, and the mixture was slowly warmed to room temperature. After the completion of the reaction as monitored by TLC, MeOH was added to quench the excess sodium hydride. The reaction was diluted with CH_2Cl_2, successively washed with H_2O and brine and dried over anhydrous Na_2SO_4. The residue was purified by flash column chromatography (hexanes/EtOAc=15:1~3:1) to afford the compound 54 (29.6 mg, 85% for 2 steps) as colorless syrup. R_f=0.70 (hexanes/EtOAc=3:1); ^1H NMR (400 MHZ, $CDCl_3$) δ 7.39-7.18 (75H, m), 5.24 (1H, m), 5.14 (1H, m), 5.03-4.83 (7H, m), 4.79-4.64 (6H, m), 4.60-4.35 (19H, m), 4.24-4.20 (2H, m), 4.06-3.92 (7H, m), 3.90-3.82 (3H, m), 3.75 (2H, m), 3.69-3.50 (12H, m), 3.47-3.38 (2H, m), 3.29 (1H, dd, J=9.4 Hz, J=9.4 Hz), 3.21-3.15 (2H, m), 1.08-1.03 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.07, 139.03, 138.84, 138.67, 138.61, 138.55, 138.53, 138.38, 138.20, 137.94, 137.91, 136.96, 128.50, 128.44, 128.37, 128.32, 128.27, 128.24, 128.21, 128.18, 128.15, 128.05, 127.99, 127.91, 127.75, 127.71, 127.67, 127.65, 127.60, 127.57, 127.51, 127.46, 127.40, 127.29, 127.26, 127.15, 127.01, 101.49, 101.34, 100.46, 98.52, 98.07, 82.18, 80.94, 80.27, 79.88, 79.49, 78.94, 77.26, 75.16, 75.05, 74.91, 74.84, 74.77, 74.66, 74.62, 74.50, 74.30, 74.20, 73.83, 73.42, 73.30, 73.18, 72.98, 72.40, 72.30, 72.13, 72.01, 71.59, 71.48, 70.74, 69.95, 68.99, 68.41, 66.32, 65.81, 62.45, 29.73, 18.09, 18.03, 12.06; MALDI-TOF: $[M+Na]^+$ calcd for $C_{14}H_{161}N_3NaO_{25}Si^+$, 2384.94; found, 2384.08.

Benzyl 2,3,4-tri-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (55)

[0253]



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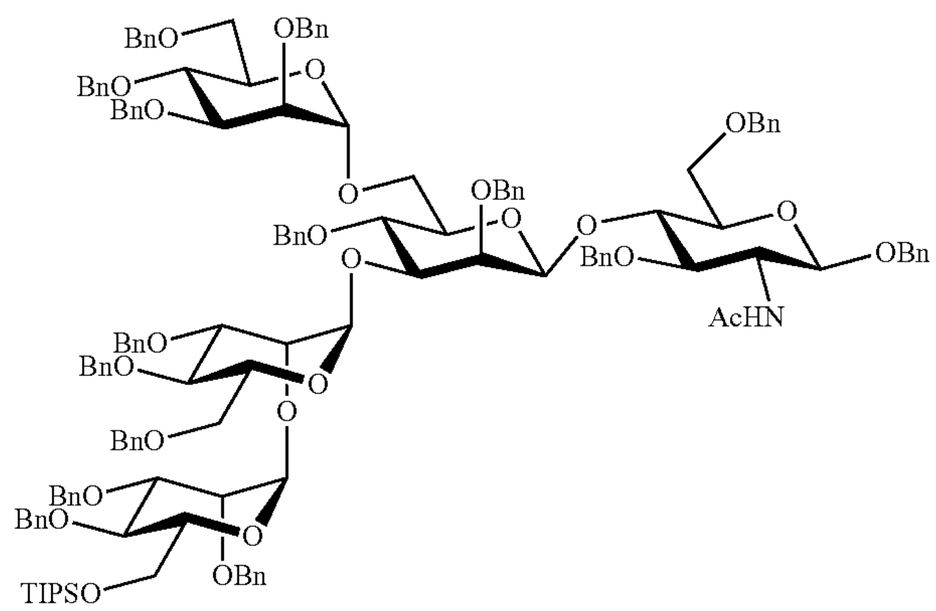
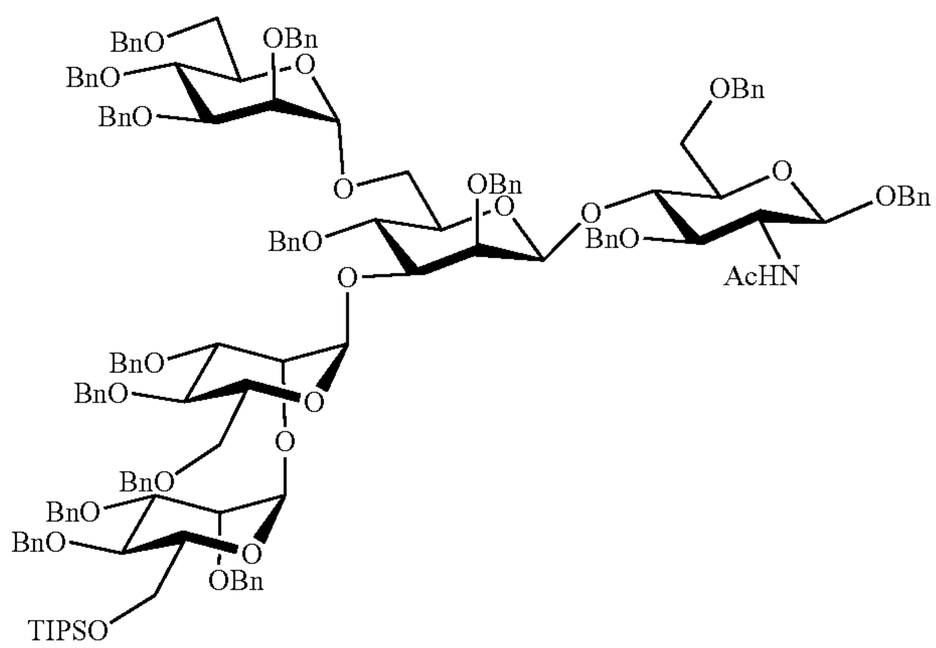
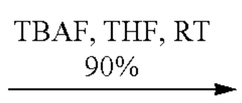


[0254] A solution of compound 54 (29.6 mg, 0.013 mmol) in a mixture of AcSH/pyridine/$CHCl_3$ (0.3 mL/0.2 mL/0.3 mL) was stirred at 60° ° C. for 23 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=6:1~2:1) to afford compound 55 (25.0 mg, 84%) as colorless syrup. R_f=0.30 (hexanes/EtOAc=2:1); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 7.36-7.13 (75H, m), 5.23 (1H, m), 5.17-5.12 (2H, m), 5.03 (1H, d, J=11.9 Hz), 4.96-4.91 (3H, m), 4.90-4.80 (4H, m), 4.76-4.74 (2H, m), 4.69 (1H, d, J=10.7 Hz), 4.65 (1H, m), 4.61-4.42 (19H, m), 4.42-4.37 (2H, m), 4.31 (1H, d, J=12.0 Hz), 4.21 (1H, dd, J=9.6 Hz, J=9.6 Hz), 4.08-3.99 (5H, m), 3.94-3.89 (3H, m), 3.88-3.84 (2H, m), 3.79 (1H, dd, J=9.7 Hz, J=9.7 Hz), 3.74 (2H, m), 3.70-3.62 (9H, m), 3.59-3.56 (1H, m), 3.55-3.53 (1H, m), 3.51-3.49 (2H, m), 3.34 (1H, q, J=7.4 Hz), 3.27 (1H, m), 3.21 (1H, d, J=10.8 Hz), 1.49 (3H, s), 1.08-1.01 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.07, 139.09, 138.96, 138.84, 138.81, 138.70, 138.67, 138.57, 138.54, 138.35, 138.14, 138.09, 137.97, 137.91, 128.42, 128.38, 128.30, 128.27, 128.23, 128.19, 128.14, 128.05, 128.02, 127.97, 127.81, 127.75, 127.71, 127.70, 127.65, 127.61, 127.56, 127.49, 127.43, 127.28, 101.45, 100.92, 99.16, 98.56, 97.99, 82.06, 80.29, 79.52, 78.87, 77.77, 77.26, 75.33, 75.12, 75.03, 74.90, 74.72, 74.22, 73.91, 73.81, 73.42, 73.19, 72.95, 72.40, 72.29, 72.14, 71.87, 71.82, 71.62, 70.83, 69.71, 68.99, 68.85, 66.56, 62.50, 56.12, 31.96, 29.73, 23.29, 22.72, 18.09, 18.03, 12.06; MALDI-TOF: $[M+Na]^+$ calcd for $C_{146}H_{165}NNaO_{26}Si^+$, 2399.13; found, 2398.92.

Benzyl 2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O)-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (56)

[0255]





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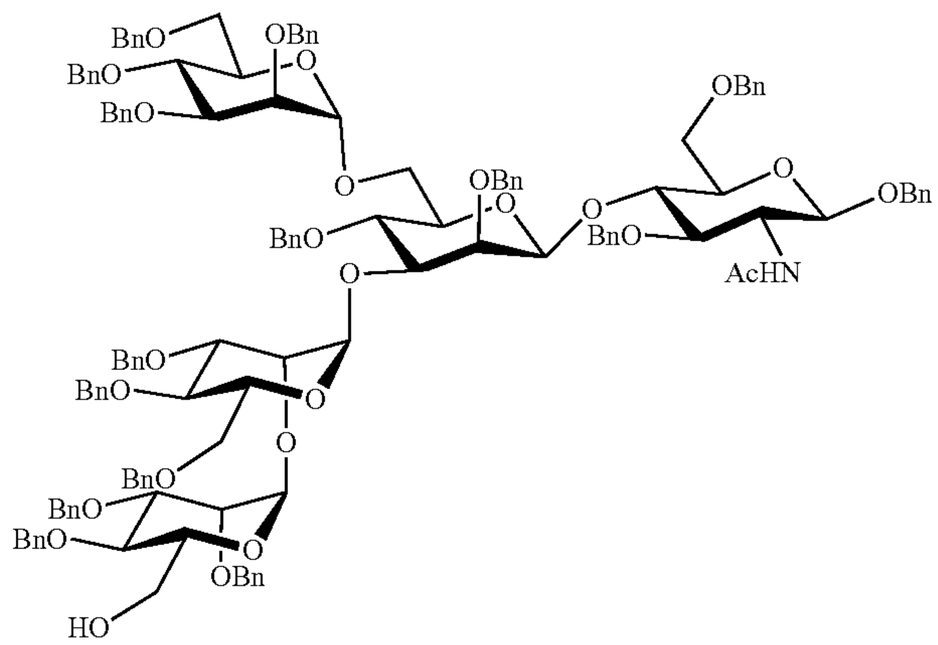
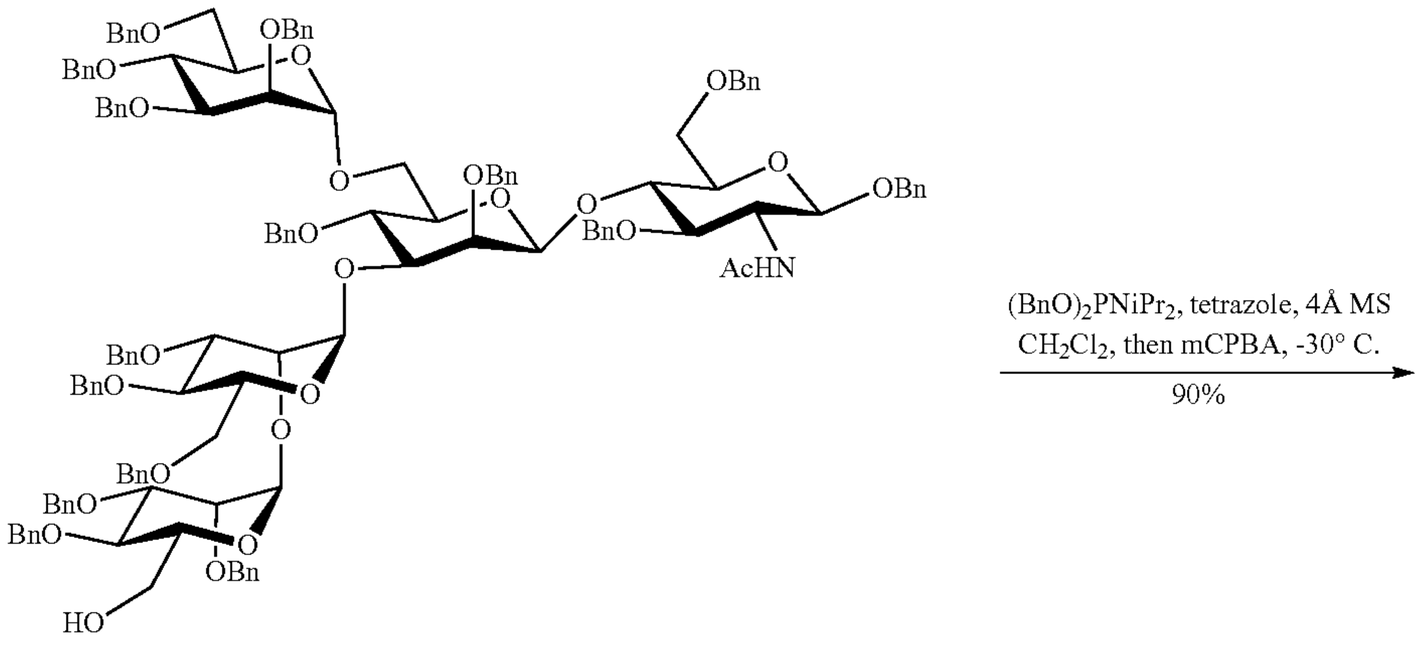


[0256] To a solution of compound 55 (25.0 mg, 0.011 mmol) in THF (1.0 mL) was added TBAF (1 M in THF, 84.2 μL), and the mixture was stirred at room temperature for 18 h. After the completion of the reaction as monitored by TLC, the resulting mixture was concentrated and subjected to flash chromatography on silica gel (hexanes/EtOAc=4:1~1:1) to afford compound 56 (21.0 mg, 90%) as colorless syrup. R_f=0.10 (hexanes/EtOAc=2:1); 1H NMR (400 MHZ, $CDCl_3$) δ 7.37-7.15 (75H, m), 5.20 (1H, m), 5.15 (1H, d, J=7.5 Hz), 5.03-5.01 (2H, m), 4.96-4.82 (7H, m), 4.76 (1H, d, J=11.8 Hz), 4.71 (1H, d, J=11.7 Hz), 4.63-4.40 (21H, m), 4.33 (1H, d, J=12.1 Hz), 4.08-3.96 (4H, m), 3.95-3.86 (5H, m), 3.85-3.73 (6H, m), 3.72-3.63 (6H, m), 3.58-3.50 (5H, m), 3.40-3.33 (1H, m), 3.32-3.25 (2H, m), 1.95 (1H, m), 1.51 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) § 170.04, 139.33, 138.92, 138.82, 138.69, 138.65, 138.58, 138.56, 138.49, 138.39, 138.37, 138.19, 138.09, 137.98, 137.89, 128.53, 128.46, 128.36, 128.31, 128.27, 128.22, 128.21, 128.19, 128.15, 127.99, 127.81, 127.79, 127.70, 127.60, 127.51, 127.47, 127.43, 127.33, 101.26, 100.91, 100.09, 99.17, 98.04, 82.19, 80.28, 79.62, 79.53, 78.85, 77.77, 77.26, 75.77, 75.26, 75.20, 75.01, 74.92, 74.85, 74.75, 73.90, 73.47, 73.41, 73.22, 72.99, 72.80, 72.61, 72.48, 72.34, 72.27, 71.96, 71.86, 70.81, 69.54, 68.96, 68.89, 66.55, 62.21, 56.12, 29.73, 23.29; MALDI-TOF: $[M+Na]^+$ calcd for $C_{137}H_{145}NNaO_{26}^+$, 2242.99; found, 2243.15.

Benzyl 2,3,4-tri-O-benzyl-6-O-dibenzylphospho-nato-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-ben-zyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-di-O-benzyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (57)

[0257]



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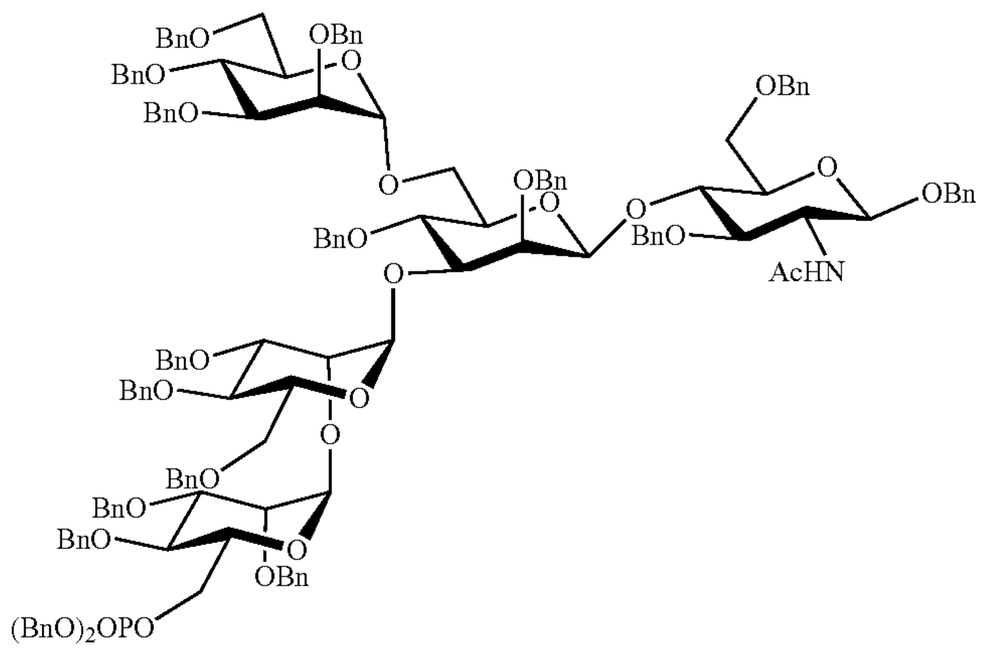
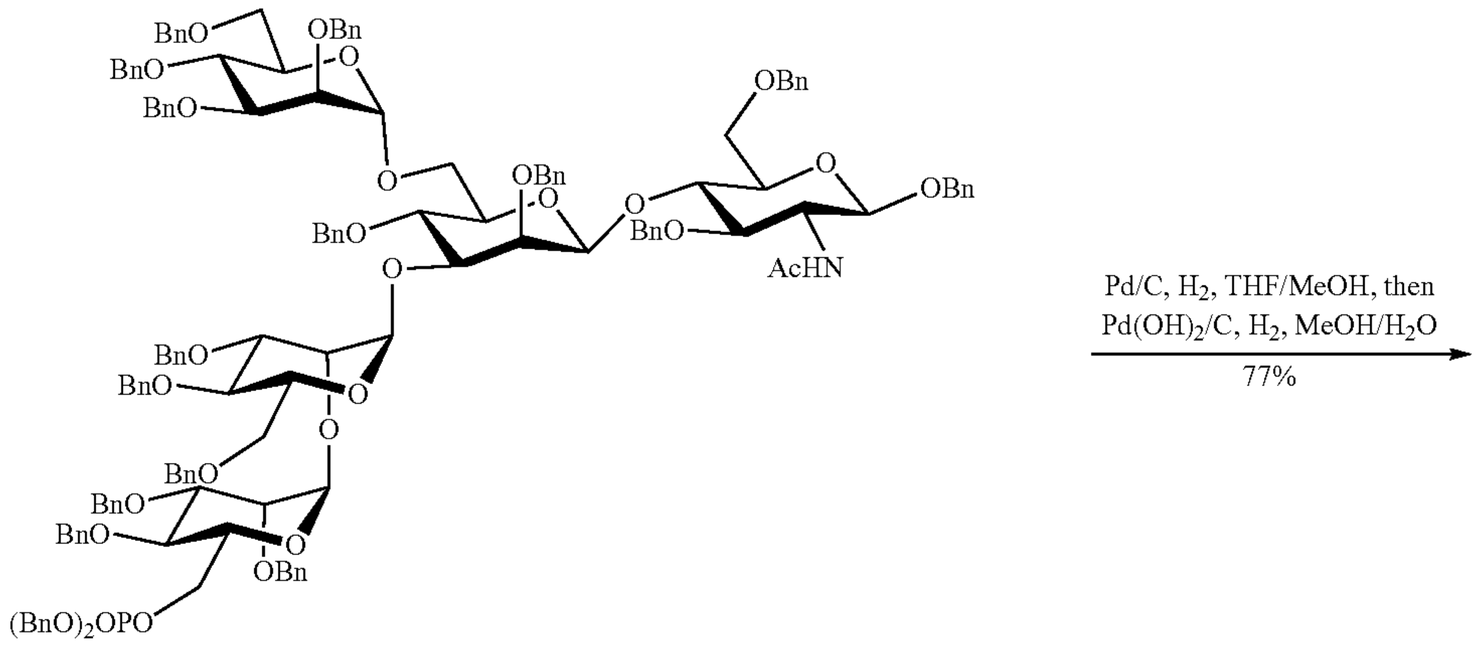


[0258] To a solution of compound 56 (21.0 mg, 0.0095 mmol) in anhydrous CH_2Cl_2 (1.0 mL) was added activated 4 Å molecular sieves (100 mg) and tetrazole (0.45 M in MeCN, 105 μL) and the mixture was stirred at room temperature for 1.5 h before $(BnO)_2PNiPr_2$ (12.8 μL) was added. The resulting mixture was further stirred overnight under argon atmosphere at room temperature until the complete disappearance of the starting material. Then the reaction was cooled to −30° C., and mCPBA (77 wt %, 11.2 mg) was added, the reaction mixture was stirred at this temperature for 1 h and then filtered through a Celite pad. The filtrate was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ (aq.), dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc=4:1~1:1) to give compound 57 (21.0 mg, 90%) as colorless syrup. R_f=0.10 (hexanes/EtOAc=2:1); ^1H NMR (400 MHZ, $CDCl_3$) δ 7.34-7.13 (85H, m), 5.28 (1H, d, J=7.7 Hz), 5.12-5.07 (2H, m), 5.05-5.02 (2H, m), 4.98-4.93 (5H, m), 4.90-4.79 (6H, m), 4.74-4.71 (3H, m), 4.60-4.58 (2H, m), 4.56-4.53 (5H, m), 4.51-4.43 (12H, m), 4.40-4.31 (3H, m), 4.15-4.13 (2H, m), 4.07-3.97 (7H, m), 3.93-3.85 (6H, m), 3.84-3.78 (3H, m), 3.75-3.68 (6H, m), 3.60-3.55 (4H, m), 3.52-3.51 (1H, m), 3.44 (1H, q, J=7.5 Hz), 3.39-3.34 (1H, m), 3.26 (1H, m), 1.50 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.63, 138.74, 138.44, 138.36, 138.21, 138.14, 138.04, 137.97, 137.91, 137.84, 137.74, 137.61, 137.51, 137.42, 135.51, 135.46, 135.41, 135.36, 132.59, 129.17, 128.22, 128.06, 127.99, 127.97, 127.88, 127.87, 127.83, 127.82, 127.79, 127.74, 127.70, 127.67, 127.57, 127.55, 127.50, 127.42, 127.35, 127.30, 127.25, 127.24, 127.17, 127.09, 127.03, 126.99, 126.93, 126.89, 126.78, 126.70, 100.67, 100.33, 98.96, 98.65, 97.52, 81.75, 79.78, 79.23, 79.05, 78.53, 77.00, 74.91, 74.77, 74.71, 74.53, 74.49, 74.36, 74.27, 74.24, 74.18, 74.12, 73.57, 73.12, 72.86, 72.84, 72.69, 72.26, 71.90, 71.82, 71.72, 71.58, 71.44, 71.33, 70.21, 69.00, 68.82, 68.69, 68.65, 68.61, 68.57, 68.40, 66.87, 66.83, 66.24, 66.04, 54.88, 29.21, 22.70; 31P NMR (146 MHZ, $CDCl_3$) δ −1.33; MALDI-TOF: $[M+Na]^+$ calcd for $C_{151}H_{158}NNaO_{29}P^+$, 2503.05; found, 2502.94.

6-O-phosphonato-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-αβ-D-glucopyranoside (58)

[0259]



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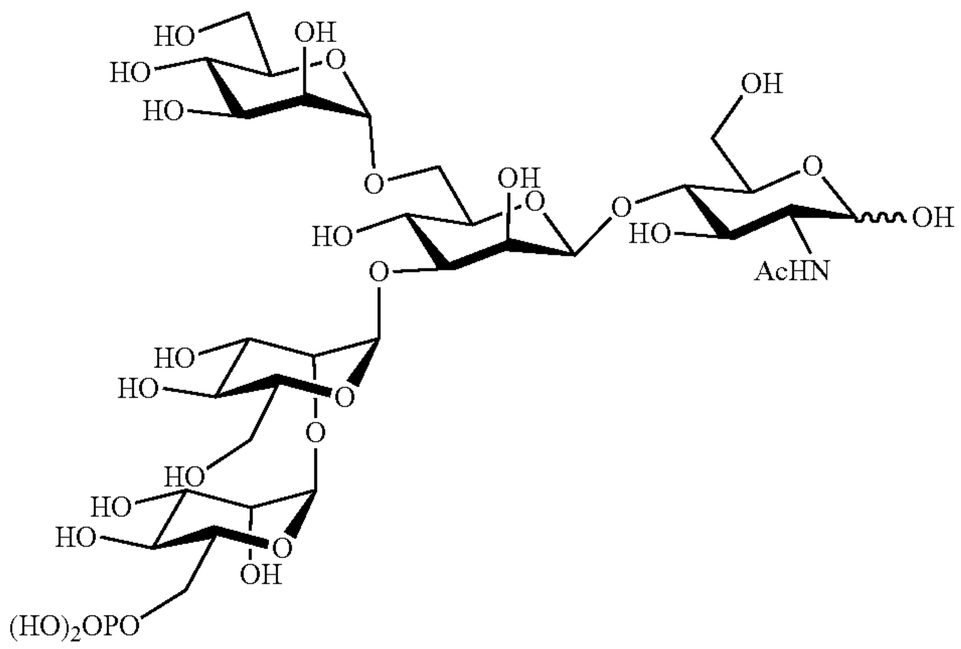
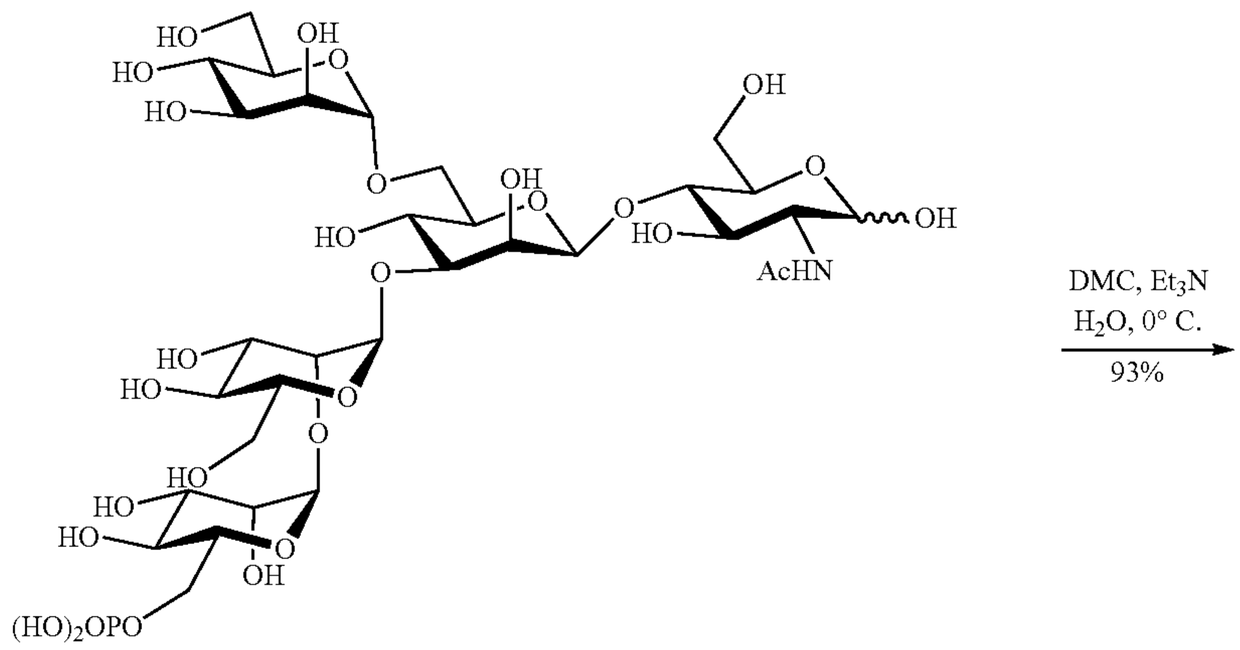


[0260] A mixture of compound 57 (44.6 mg, 0.018 mmol) and Pd/C (10 wt. % loading, 20 mg) in MeOH (1.5 mL) and THF (1.5 mL) was stirred under H_2 atmosphere for 21 h. The reaction mixture was filtered through a Celite pad, then concentrated to dryness. The mixture of the residue and $Pd(OH)_2/C$ (20 wt. % loading, 30 mg) in MeOH (2.0 mL) and H_2O (2.0 mL) was stirred under H_2 atmosphere for further 22 h. The reaction mixture was filtered through a Celite pad. The filtrate was concentrated to dryness then dissolved in H_2O and lyophilized. The crude product was purified on a Sephadex G-10 column by elution with H_2O. Fractions containing the product were pooled and lyophilized to give compound 58 (13.1 mg, 77%) as white solid. R_f=0.30 (n-BuOH/EtOH/H_2O/AcOH=1:1:1:0.05); ^{1}H NMR (400 MHZ, D_2O) δ 5.34 (1.00H, m), 5.15 (1H, d, J=3.2 Hz), 4.97 (1.14H, m), 4.87 (1.16H, m), 4.76-4.75 (1.80H, m), 4.66-4.65 (1.00H, m), 4.17-4.15 (1.19H, m), 4.03-4.01 (2.51H, m), 3.99-3.96 (1.55H, m), 3.95-3.89 (3.98H, m), 3.88-3.76 (11.96H, m), 3.74-3.64 (11.78H, m), 3.62-3.55 (3.85H, m), 1.99 (3.00H, m); ^{13}C NMR (100 MHZ, D_2O) δ 174.42, 174.13, 102.00, 100.33, 99.73, 99.26, 94.50, 90.07, 79.69, 79.63, 79.29, 78.28, 74.11, 73.75, 73.01, 72.27, 72.12, 72.07, 71.82, 69.99, 69.92, 69.77, 69.56, 69.51, 68.65, 66.60, 66.45, 66.09, 65.85, 65.62, 65.53, 63.10, 60.68, 60.56, 59.76, 59.63, 55.67, 53.20, 21.85, 21.54; 31P NMR (146 MHz, D_2O) δ 2.94 (overlapped signals); HRMS: $[M+H]^+$ calcd for $C_{32}H_{57}NO_{29}P^+$, 950.2748; found, 950.2755.

2-Methyl-[6-O-phosphonato-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline (10)

[0261]



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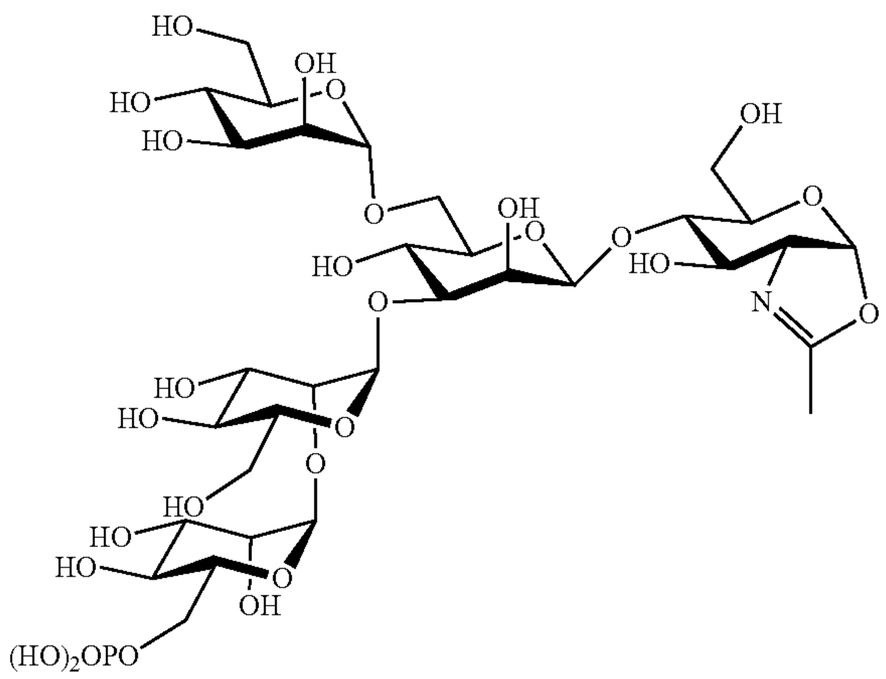
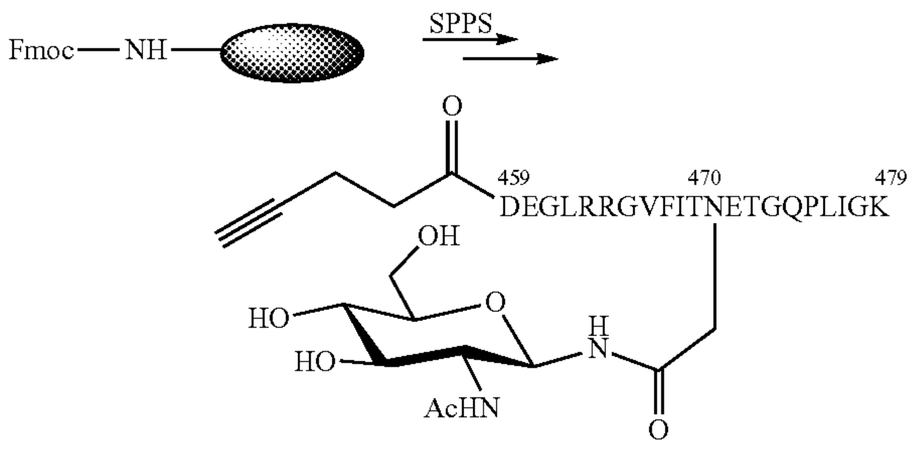


[0262] To a solution of compound 58 (8.0 mg, 0.0084 mmol) in H_2O (250 μL) were added Et_3N (47.2 μL) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC, 28.6 mg) at 0° C. The reaction mixture was monitored by DIONEX HPAEC-PAD. After 2 h, the HPAEC analysis indicated that the free oligosaccharide was converted into a new oligosaccharide that was eluted earlier than the reducing sugar under the HPAEC condition (see general method). The product was purified by gel filtration on a Sephadex G-10 column that was eluted with 0.1% aq Et_3N to afford compound 10 (7.3 mg, 93%) as white solid after lyophilization with 5 mol. % of NaOH. 1H NMR (400 MHZ, D_2O) δ 6.01 (1H, d, J=7.3 Hz), 5.32 (1H, m), 4.94 (1H, m), 4.88 (1H, m), 4.32-4.31 (1H, m), 4.12-4.11 (1H, m), 4.04-3.98 (3H, m), 3.95-3.85 (6H, m), 3.84-3.80 (2H, m), 3.79-3.73 (5H, m), 3.71-3.62 (8H, m), 3.61-3.55 (4H, m), 3.35-3.32 (1H, m), 1.99 (3H, d, J=1.7 Hz); ^{13}C NMR (100 MHZ, D_2O) δ 167.65, 102.48, 101.25, 100.69, 99.92, 99.59, 80.03, 78.74, 77.78, 74.31, 73.33, 72.68, 72.59, 70.96, 70.50, 70.37, 70.13, 69.98, 69.95, 69.89, 69.05, 66.95, 66.84, 66.43, 66.20, 65.73, 65.03, 62.99, 61.67, 61.00, 60.94, 12.95; 31P NMR (146 MHz, D_2O) δ 4.02; HRMS: $[M+H]^+$ calcd for $C_{32}H_{55}NO_{28}P^+$, 932.2643; found, 932.2669.

Example 10: Synthesis of Glycopeptides

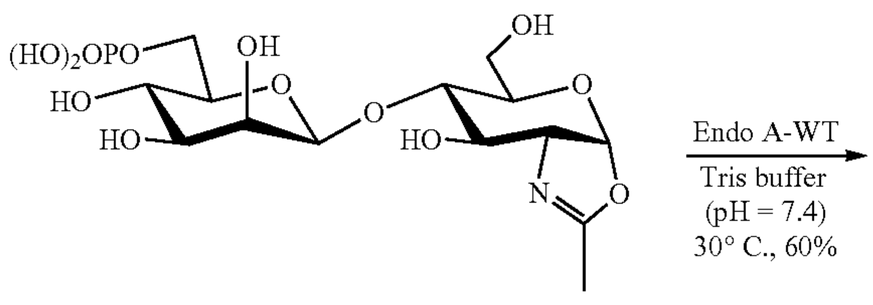
[0263] GlcNAc-Peptide Derived from rhGAA Containing N470 Glycosite



[0264] The GlcNAc-Peptide was obtained from SPPS. Synthesis was based on Fmoc chemistry using Rink Amide AM resin (0.66 mmol/g) on a 0.1 mmol scale. Couplings were performed using 5 equiv. of Fmoc-protected amino acids, 5 equiv. of HOBT and 5 equiv. of DIC in DMF. The GlcNAc-Asn building block (3 equiv.) was coupled to the growing peptide at 90° ° C. with a 50 Hz MW power for 10 min, Fmoc-Arg(Pbf)-OH was double coupled (RT without MW for 25 min, followed by 90° C. with 50 Hz MW power for 2 min), and all other amino acids were coupled at 90° ° C. with 50 Hz MW power for 2 min. Fmoc deprotection was carried out with 20% piperidine in DMF containing 0.1 M HOBt. Upon completion of the sequence, 4-pentynoic acid was coupled at the N-terminus to install the alkyne group. The resin was washed with DMF (3×) and DCM (3×) then cleavage was carried out using cocktail R (TFA/Thioanisole/Ethanedithiol/Anisole=90/5/3/2) treatment for 2 h. The resin was then filtered and the solution was added to cold diethyl ether for precipitation. The crude peptide was purified on preparative RP-HPLC to afford the peptide (99.1 mg, 38% yield over all steps). ESI-MS: Calcd., M=2583.89; found (m/z): 646.90 $[M+4H]^{4+}$+, 861.43 $[M+3H]^{3+}$, 1292.36 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=2583.4; RP-HPLC retention time, t_R=18.7 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).

Glycopeptide 59

[0265]



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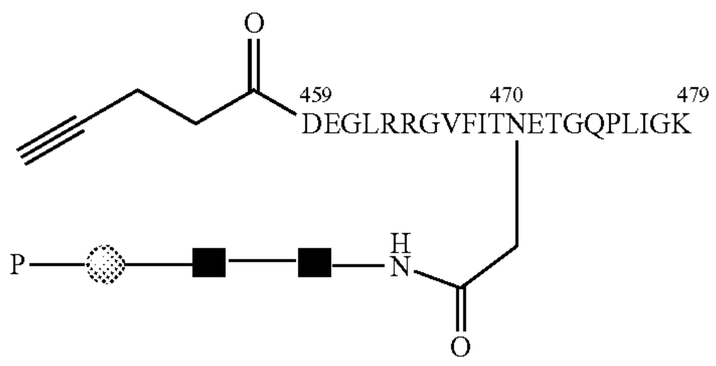
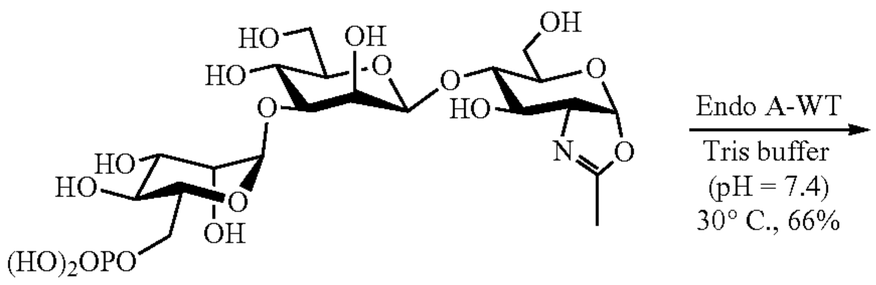


59

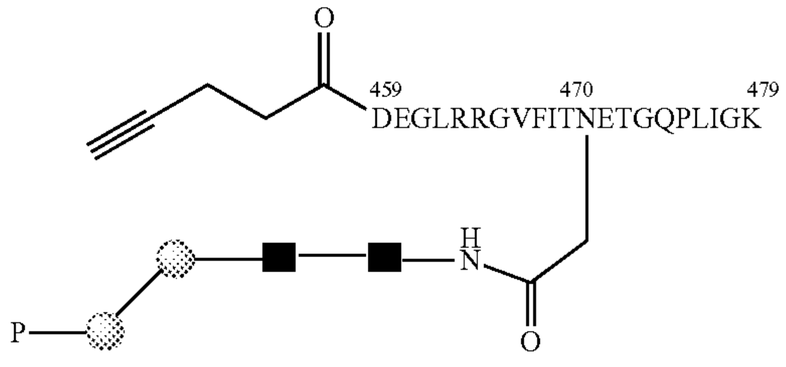
[0266] GlcNAc-peptide (3.0 mg, 1.16 μmol) was incubated at 30° C. together with oxazoline 5 (2.1 mg, 4 eq) and Endo A-WT (120 μg) in Tris buffer (100 mM, pH 7.4, 100 μL). The reaction was monitored by analytical RP-HPLC. Upon completion of the transglycosylation, the reaction was quenched using 0.1% aq. TFA and purified by RP-HPLC (gradient, 10-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 4.0 mL/min) to give glycopeptides 59 (2.1 mg, 60%) as white solid. ESI-MS: Calcd., M=3029.20; found (m/z): 758.13 $[M+4H]^{4+}$, 1010.14 $[M+3H]^{3+}$, 1515.15 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=3028.7; RP-HPLC retention time, t_R=19.5 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).

Glycopeptide 60

[0267]



7

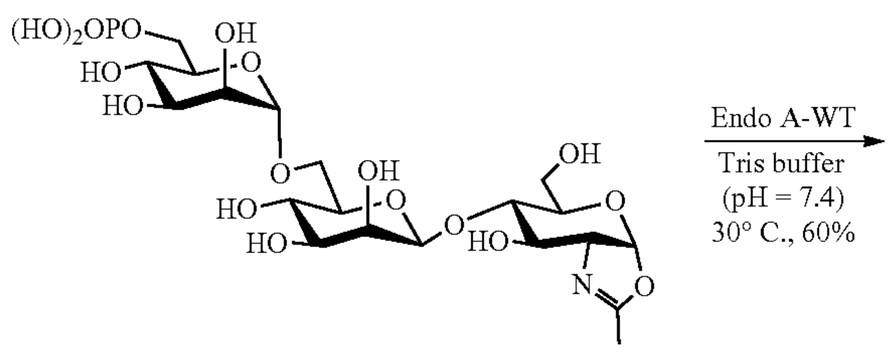


60

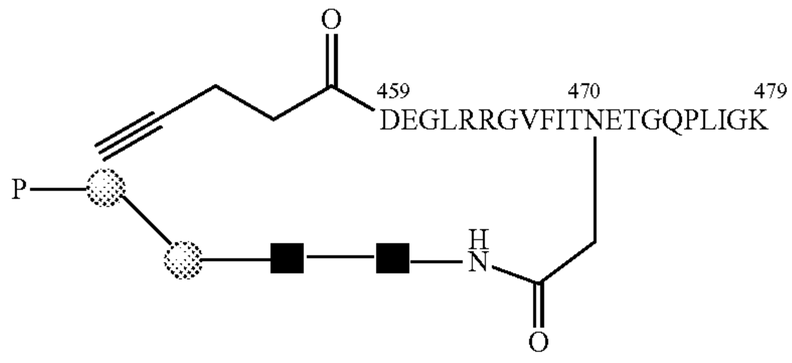
[0268] GlcNAc-peptide (3.0 mg, 1.16 μmol) was incubated at 30° C. together with oxazoline 7 (3.5 mg, 5 eq) and Endo A-WT (120 μg) in Tris buffer (100 mM, pH 7.4, 100 μL). The reaction was monitored by analytical RP-HPLC. Upon completion of the transglycosylation, the reaction was quenched using 0.1% aq. TFA and purified by RP-HPLC (gradient, 10-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 4.0 mL/min) to give glycopeptides 60 (2.4 mg, 66%) as white solid. ESI-MS: Calcd., M=3191.34; found (m/z): 798.74 $[M+4H]^{4+}$, 1064.61 $[M+3H]^{3+}$, 1596.46 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=3190.9; RP-HPLC retention time, t_R=19.4 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).

Glycopeptide 61

[0269]



6

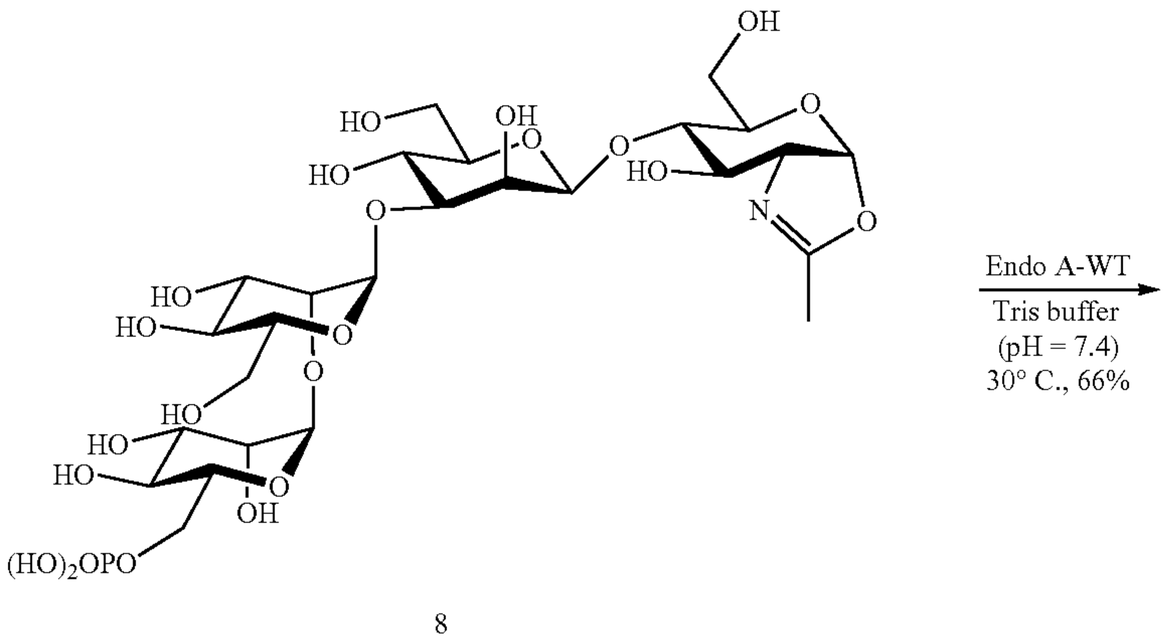


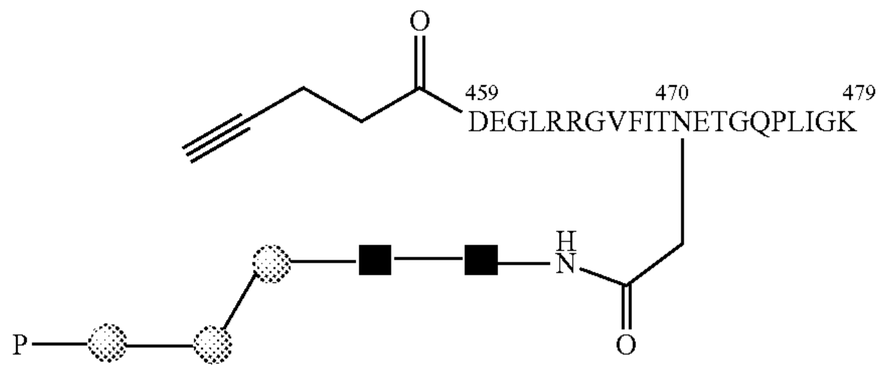
61

[0270] GlcNAc-peptide (3.0 mg, 1.16 μmol) was incubated at 30° C. together with oxazoline 6 (2.8 mg, 4 eq) and Endo A-WT (60 μg) in Tris buffer (100 mM, pH 7.4, 100 μL). The reaction was monitored by analytical RP-HPLC. Upon completion of the transglycosylation, the reaction was quenched using 0.1% aq. TFA and purified by RP-HPLC (gradient, 10-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 4.0 mL/min) to give glycopeptides 61 (2.2 mg, 60%) as white solid. ESI-MS: Calcd., M=3191.34; found (m/z): 798.74 $[M+4H]^{4+}$, 1064.86 $[M+3H]^{3+}$, 1596.58 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=3190.4; RP-HPLC retention time, t_R=19.2 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).

Glycopeptide 62

[0271]



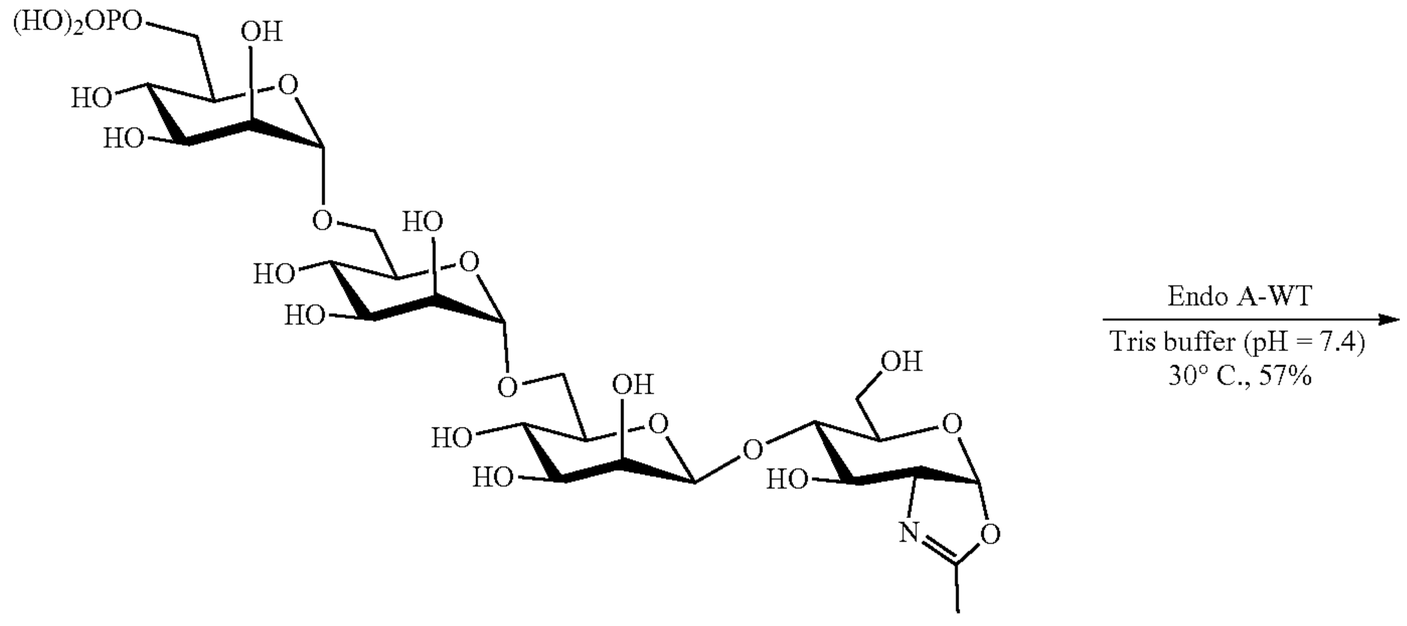


62

[0272] GlcNAc-peptide (3.0 mg, 1.16 μmol) was incubated at 30° C. together with oxazoline 8 (4.2 mg, 5 eq) and Endo A-WT (60 μg) in Tris buffer (100 mM, pH 7.4, 100 μL). The reaction was monitored by analytical RP-HPLC. Upon completion of the transglycosylation, the reaction was quenched using 0.1% aq. TFA and purified by RP-HPLC (gradient, 10-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 4.0 mL/min) to give glycopeptides 62 (2.6 mg, 66%) as white solid. ESI-MS: Calcd., M=3353.48; found (m/z): 839.27 $[M+4H]^{4+}$, 1118.50 $[M+3H]^{3+}$, 1677.12 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=3352.7; RP-HPLC retention time, t_R=19.2 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).

Glycopeptide 63

[0273]



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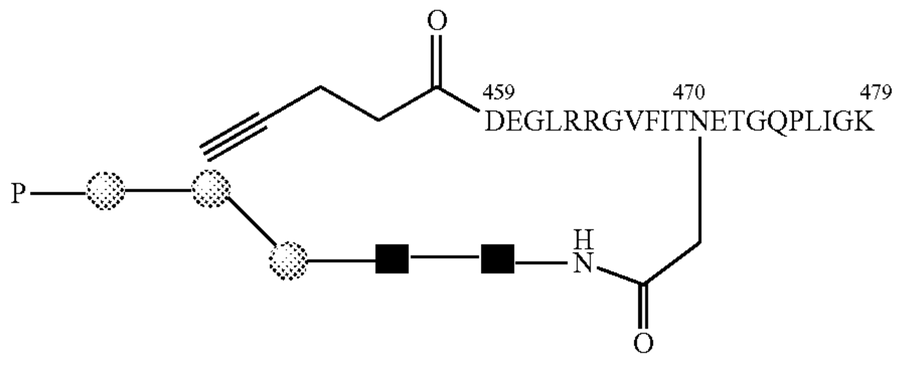
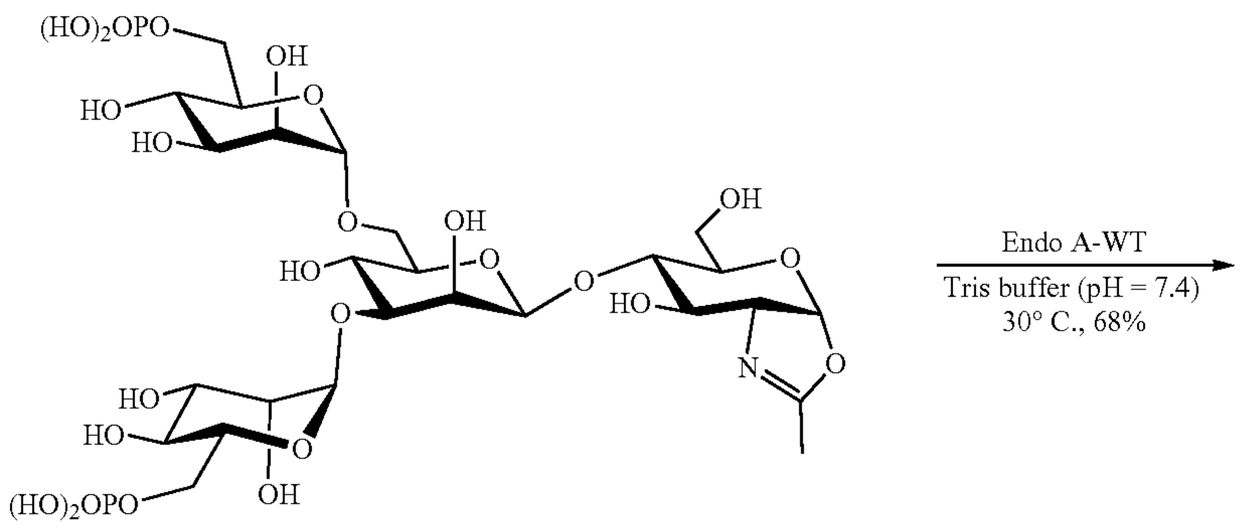


63

[0274] GlcNAc-peptide (3.0 mg, 1.16 μmol) was incubated at 30° C. together with oxazoline 9 (4.8 mg, 6 eq) and Endo A-WT (120 μg) in Tris buffer (100 mM, pH 7.4, 100 μL). The reaction was monitored by analytical RP-HPLC. Upon completion of the transglycosylation, the reaction was quenched using 0.1% aq. TFA and purified by RP-HPLC (gradient, 10-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 4.0 mL/min) to give glycopeptides 63 (2.2 mg, 57%) as white solid. ESI-MS: Calcd., M=3353.48; found (m/z): 839.25 $[M+4H]^{4+}$, 1118.85 $[M+3H]^{3+}$, 1677.48 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=3353.1; RP-HPLC retention time, t_R=19.2 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).

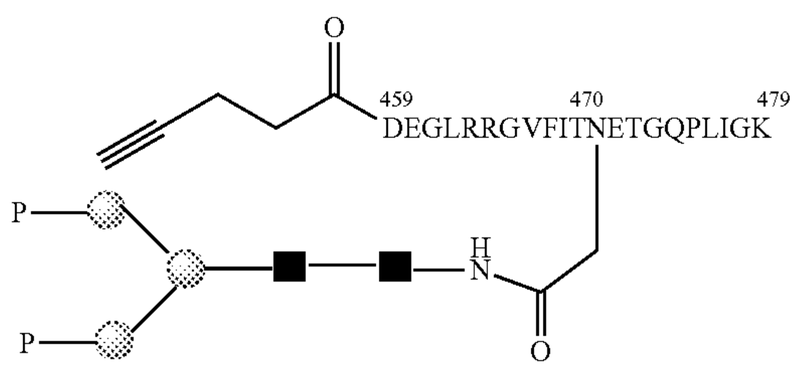
Glycopeptide 64

[0275]



4

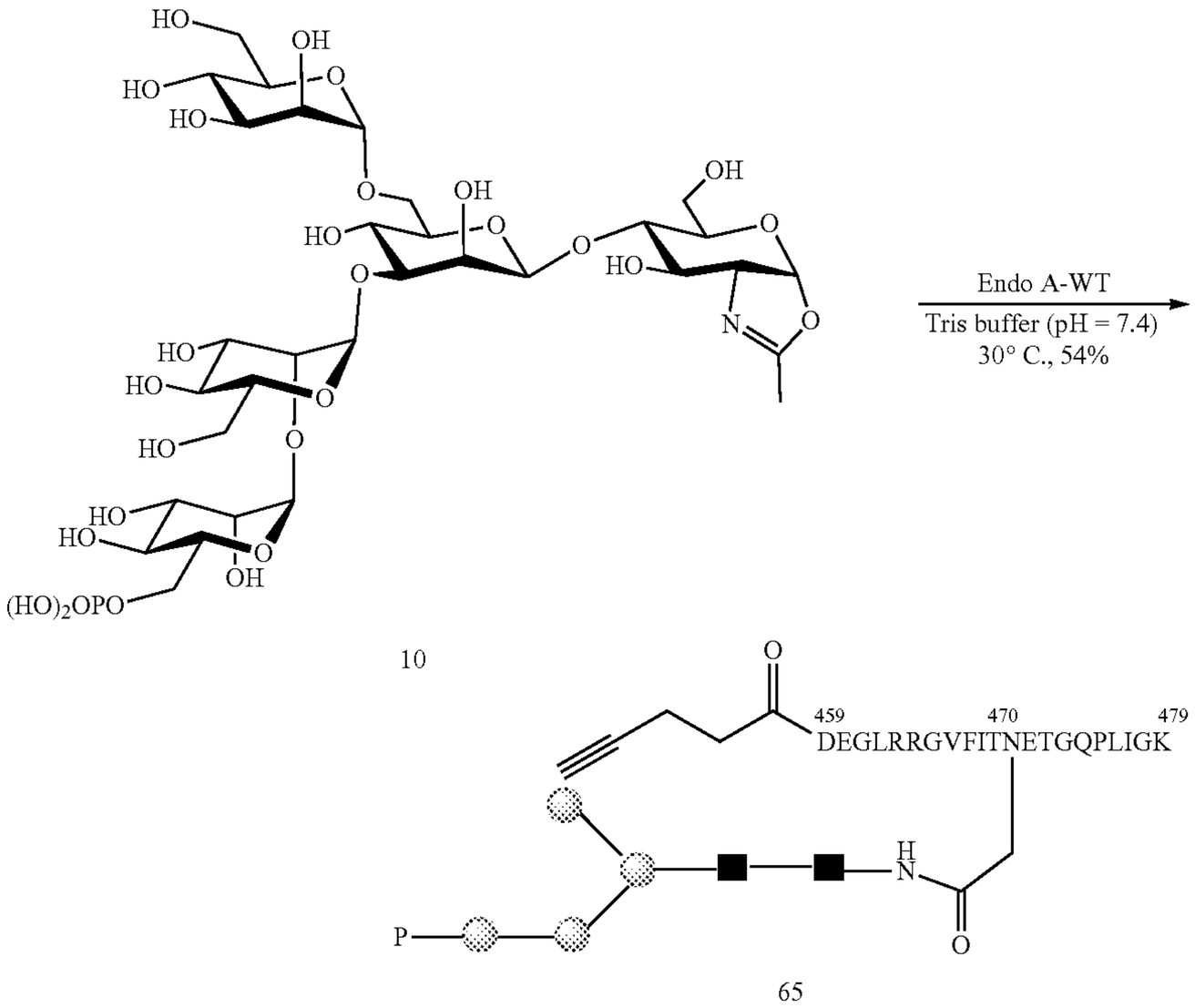
[0276] GlcNAc-peptide (3.0 mg, 1.16 μmol) was incubated at 30° C. together with oxazoline 4[7] (4.9 mg, 5 eq) and Endo A-WT (100 μg) in Tris buffer (100 mM, pH 7.4, 100 μL). The reaction was monitored by analytical RP-HPLC. Upon completion of the transglycosylation, the reaction was quenched using 0.1% aq. TFA and purified by RP-HPLC (gradient, 10-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 4.0 mL/min) to give glycopeptides 64 (2.7 mg, 68%) as white solid. ESI-MS: Calcd., M=3433.46; found (m/z): 859.25 $[M+4H]^{4+}$, 1145.02 $[M+3H]^{3+}$, 1717.00 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=3432.9; RP-HPLC retention time, t_R=20.2 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).



64

Glycopeptide 65

[0277]

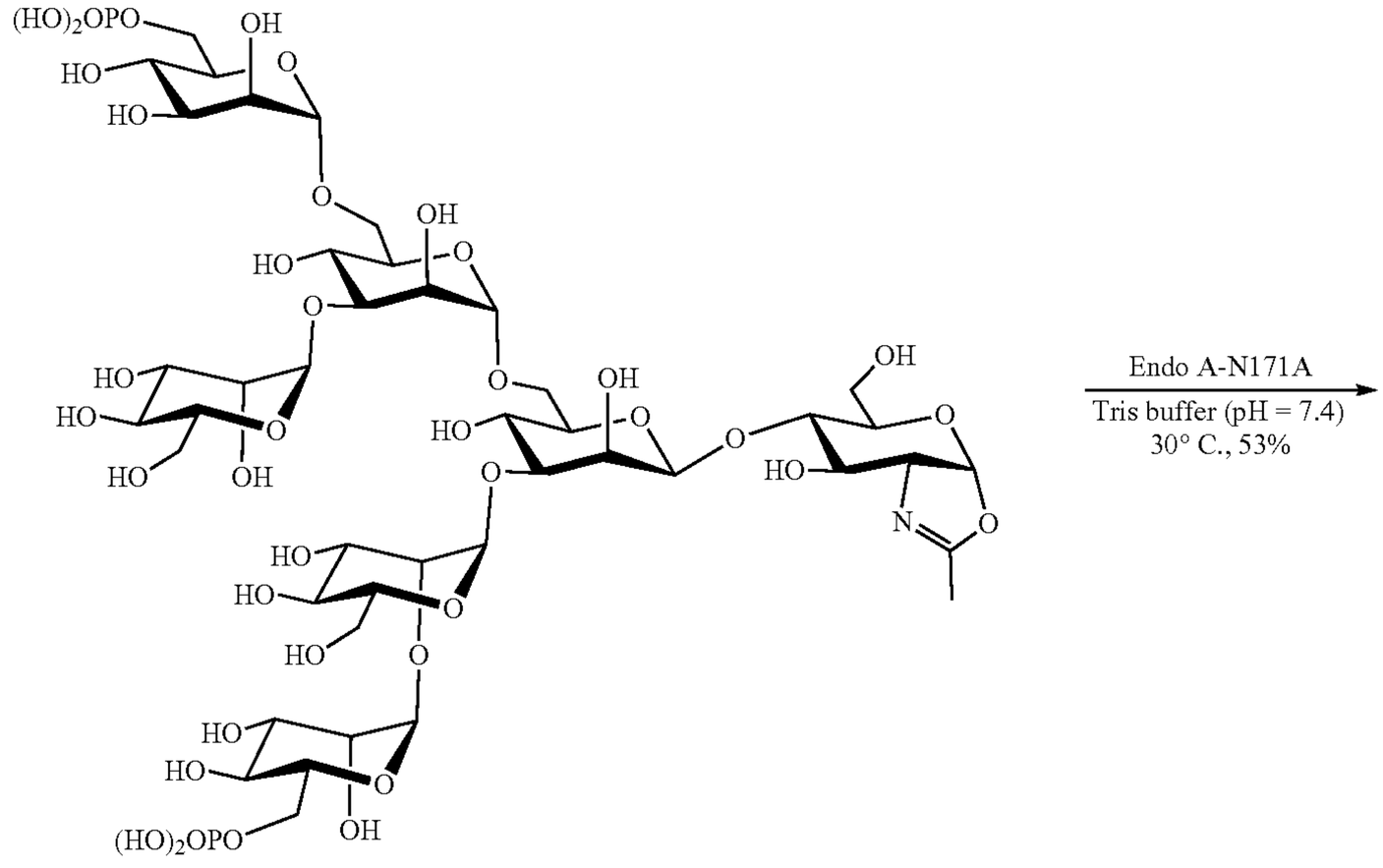


65

[0278] GlcNAc-peptide (3.0 mg, 1.16 μmol) was incubated at 30° C. together with oxazoline 10 (5.4 mg, 5 eq) and Endo A-WT (120 μg) in Tris buffer (100 mM, pH 7.4, 100 μL). The reaction was monitored by analytical RP-HPLC. Upon completion of the transglycosylation, the reaction was quenched using 0.1% aq. TFA and purified by RP-HPLC (gradient, 10-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 4.0 mL/min) to give glycopeptides 65 (2.2 mg, 54%) as white solid. ESI-MS: Calcd., M=3515.62; found (m/z): 879.89 $[M+4H]^{4+}$, 1172.78 $[M+3H]^{3+}$, 1758.44 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=3515.6; RP-HPLC retention time, t_R=19.1 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).

Glycopeptide 66

[0279]



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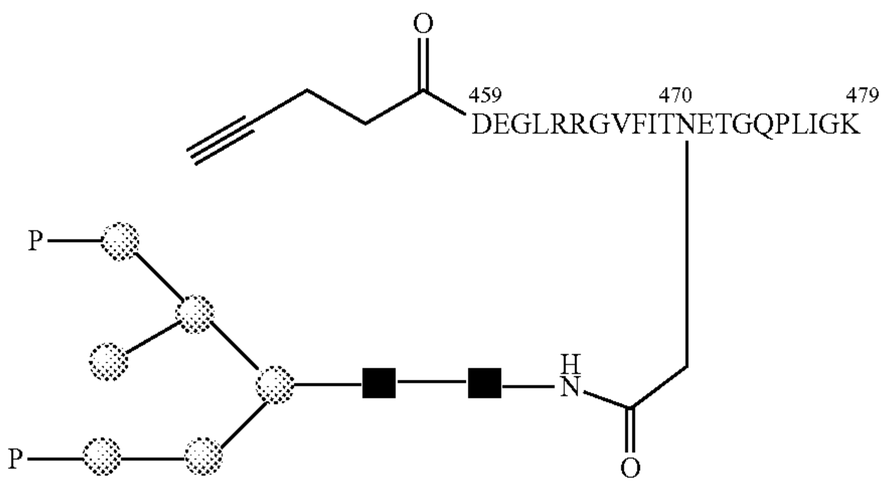


[0280] GlcNAc-peptide (2.0 mg, 0.77 μmol) was incubated at 30° C. together with phosphorylated oxazoline 1[7] (5.2 mg, 5 eq) and Endo A-N171A (180 μg) in Tris buffer (100 mM, pH 7.4, 100 μl). The reaction was monitored by analytical RP-HPLC. Upon completion of the transglycosylation, the reaction was quenched using 0.1% aq. TFA and purified by RP-HPLC to give glycopeptides 66 (1.54 mg, 53%). ESI-MS: Calcd., M=3919.88; found (m/z): 980.88 $[M+4H]^{4+}$, 1307.60 $[M+3H]^{3+}$, 1960.92 $[M+2H]^{2+}$. Deconvolution of the ESI-MS: M=3919.5; RP-HPLC retention time, t_R=19.9 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min).

Example 11: Glycan Remodeling

Glycoprotein 68

[0281] Stepwise strategy. RNase B (5.8 mg) was treated with wild-type Endo A (66 μg) in PBS buffer (pH=7.2, 580 μL) at 37° C. for 1 h. Upon the completion of the reaction as monitored by analytical RP-HPLC, the reaction was purified by preparative HPLC to give the homogeneous GlcNAc-RNase B 67 (4.6 mg, 86%). ESI-MS: Calcd., M=13886; found (m/z): 1157.93 $[M+12H]^{12+}$, 1263.28 $[M+11H]^{11+}$, 1389.51 $[M+10H]^{10+}$, 1543.78 $[M+9H]^{9+}$, 1736.63 $[M+8H]^{8+}$. Deconvolution of the ESI-MS: M=13886 (FIG. **15**).

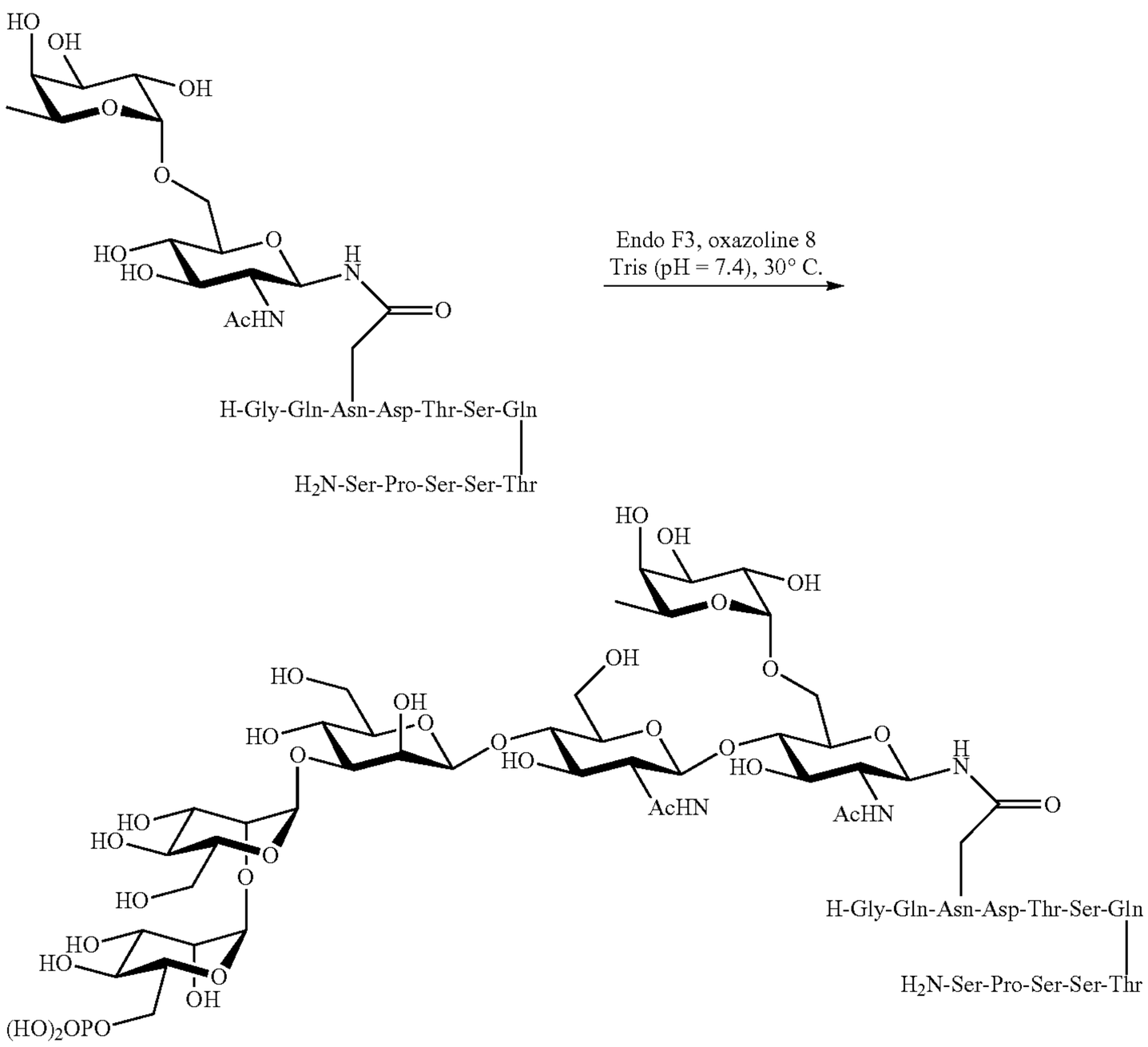
[0282] Then to a solution of oxazoline 8 (500 μg, 0.65 μmol, 9 eq) and GlcNAc-RNase B 67 (1.0 mg, 0.072 μmol) in PBS buffer (150 mM, pH 7.2, 20 μL) was added Endo A-WT (200 μg) at 30° C. The reaction was monitored with analytical RP-HPLC. After 3 h, another portion of oxazoline (280 μg, 5 eq) was added and this procedure was repeated 2 to 3 times until the GlcNAc-RNase B was consumed. Upon completion of the transglycosylation, the reaction was purified by RP-HPLC to give glycoprotein 68 as white solid (0.74 mg, 71%). ESI-MS: Calcd., M=14655; found (m/z): 1047.87 $[M+14H]^{14+}$, 1128.39 $[M+13H]^{13+}$, 1222.13 $[M+12H]^{12+}$, 1333.33 $[M+11H]^{11+}$, 1466.57 $[M+10H]^{10+}$, 1629.37 $[M+9H]^{9+}$, 1832.80 $[M+8H]^{8+}$. Deconvolution of the ESI-MS: M=14656. RP-HPLC retention time, t_R=17.4 min (gradient, 5-40% aq MeCN containing 0.1% FA for 30 min; flow rate, 1.0 mL/min). In this step, 0.93 mg of solid was obtained after HPLC purification, and ca.~ 80% was the desired glycoprotein 68 according to the MS spectrum, which was not separable from the starting material due to the small size of the tetrasaccharide, so the yield was calculated as follows: 930 μg*80%=744 μg (0.051 μmol), 0.051/0.072=70.5%. The same method was used in the "one-pot" strategy.

[0283] "One-pot" strategy. RNase B (1.0 mg) was incubated with Endo A-WT (200 μg) in PBS buffer (pH=7.2, 20 μL) at 30° C. for 30 min before the addition of oxazoline 8 (308 μg, 6 eq). The reaction was monitored with analytical RP-HPLC, and additional oxazoline was added to push the reaction to completion as described in the stepwise strategy. Preparative RP-HPLC afforded the desired glycoprotein 68 as white solid (0.62 mg, 64%).

"One-Pot" Glycan Remodeling of rhGAA with Wild-Type Endo A

[0284] The commercial Lumizyme (Genzyme, Sanofi) was purified by buffer exchange with PBS (150 mM, pH=7.2) to remove extra additions before use. The resulting rhGAA (0.95 mg) was incubated with Endo A-WT (100 μg) in PBS buffer (pH=7.2, 25 μL) at 30° C. for 2 h before the addition of oxazoline 8 (125 μg, 15 eq). After 30 min, another batch of oxazoline (125 μg, 15 eq) was added and this procedure was repeated 6 to 7 times to consume most of the starting material. Upon the completion of the reaction, the resulting mixture was treated with Glutathione Agarose (Thermo Fisher, resin suspended in 200 μL solution) to remove the GST-tagged Endo A-WT, and the cleaved glycans and extra salts were removed by buffer exchange (PBS×5) to get the remodeled rhGAA (820 μg, 86%).

Transglycosylation of α1,6FucGlcNAc-CD52 with Wild-Type Endo F3



[0285] To a solution of oxazoline 8 (200 μg, 0.26 μmol, 4 eq) and Fuca1,6GlcNAc-CD52 (100 μg, 0.072 μmol) in Tris buffer (100 mM, pH 7.4, 5 μL) was added Endo F3-WT (3.0 μg) at 30° C. The reaction was complete within 30 min. MALDI-TOF: $[M+H]^+$ calcd for $C_{85}H_{142}N_{18}O_{55}P^+$, 2327.12; found, 2327.65; $[M+Na]^+$ calcd for $C_{85}H_{141}N_{18}NaO_{55}P^+$, 2349.10; found, 2349.51; $[M-H+2Na]^+$ calcd for $C_{85}H_{140}N_{18}Na_2O_{55}P^+$, 2371.08; found, 2371.50; $[M-2H+3Na]^+$ calcd for $C_{85}H_{139}N_{18}Na_{3055}PT$, 2393.06; found, 2393.49; $[M-H]^-$ calcd for $C_{85}H_{140}N_{18}O_{55}P^+$, 2325.10; found, 2325.84; $[M-2H+Na]^-$ calcd for $C_{85}H_{139}N_{18}NaO_{55}P^+$, 2347.08; found, 2348.04.

"One-Pot" Glycan Remodeling of rhGAA with Wild-Type Endo F3

[0286] The commercial Lumizyme (Genzyme, Sanofi) was directly used without pretreatment (the additions such as mannitol were necessary to keep the protein soluble because Endo F3 would cleave most of the complex-type N-glycans). To the commercial mixture (2.4 mg powder, containing ~400 μg rhGAA) in PBS (pH=7.2, 10 μL) was added Endo F3-WT (40 μg) and oxazoline 8 (100 μg, 30 eq). After 2 h, another batch of oxazoline (100 μg, 30 eq) was added and this procedure was repeated twice to consume most of the starting material. Upon the completion of the reaction, the resulting mixture was treated with Histrap column (GE Healthcare, 1 mL) to remove the His-tagged Endo F3-WT, and the cleaved glycans and extra salts were removed by buffer exchange (PBS×5) to get the remodeled rhGAA (282 μg, 71%).

Example 12: Biological Studies

[0287] Surface Plasmon Resonance (SPR) Measurements. SPR measurements were performed on a Biacore T200 instrument (GE Healthcare). Recombinant human IGF-II R (CI-MPR) was purchased from R&D Systems. Approximately 7000 resonance units (RU) of CI-MPR was immobilized on a CM5 sensor chip in a sodium acetate buffer (25 μg/mL, pH 4.0) at 25° C., using the amine coupling kit provided by the manufacturer. Mannose 6-phosphates containing glycopeptides or glycoproteins were determined at 25° C. under a flow rate of 10 μL/min. HBS-P+ buffer (10 mM HEPES, 150 mM NaCl, 0.05% surfactant P20, pH 7.4) was used as sample buffer and running buffer. Association was measured for 3 min and dissociation for 10 min at the same flow rate (10 μL/min). The surface regeneration was performed by 2 M $MgCl_2$ at a flow rate of 10 μL/min for 60 s. Synthetic glycopeptide and glycoprotein analytes flowed over an immobilized chip with 2-fold serial dilution of the highest concentration of 4 μM (for glycopeptides) or 1 μM (for RNase B) or 250 nM (for rhGAA). Kinetic analyses were performed by global fitting of the binding data to a 1:1 Langmuir binding model using BIAcore T200 evaluation software.

[0288] rhGAA enzyme activity assay. The enzyme activity was assayed by using the substrate 4-methylumbelliferyl-α-D-glucopyranoside (4-MUG) (Sigma-Aldrich) which generates fluorescence on digestion.[9, 10] To a solution of 4-MUG (3.0 mM) in acetate buffer (200 μL, containing 0.2 M sodium acetate, 0.4 M potassium chloride, pH 4.3) was added 1.0 μg of rhGAA or remodeled rhGAA (~ 50 nM), and the reaction mixture was incubated at 37° C. The reaction was monitored at 0 min, 1 min, 2 min, 5 min, 10 min and 15 min by taking 20 μL of aliquot and adding 50 μL of stop buffer (100 mM glycine/NaOH, pH 11). Fluorescence was measured by a spectrophotometer with 355 nm excitation and 460 nm emission, and the error bar was based on three independent assays.

[0289] Muscle cell culture, treatment, processing, and analysis. The biological effect of the Endo-A (69) and Endo-F3 (70) remodeled rhGAA was investigated in an in vitro model of Pompe disease.[11, 12] The myoblasts are grown on Matrigel (Corning; 354234)-coated 6-well plates at 33° C. in an atmosphere of 5% CO_2 in proliferation medium [20% fetal bovine serum, 10% horse serum, 1% chick embryo extract, recombinant IFN-γ (100 U/mL; Life Technologies), 1× penicillin/streptomycin/L-glutamine in high-glucose (4.5 g/L) DMEM]. When the cells reach 70-80% confluency (3-4 days), the medium is switched to differentiation medium [DMEM containing 2% horse serum, 0.5% chick embryo extract, recombinant human insulin (10 μg/mL, Life Technologies, 12585-014), 1× penicillin/streptomycin/L-glutamine], and the cells are moved to 37° C. in an atmosphere of 5% CO_2. Myotubes begin to form within 3-4 days; they can survive for ~ 8-10 days in culture until they start twitching and detach from the surface.

[0290] The commercial rhGAA, Endo-A- or Endo-F3 remodeled rhGAA were added to the myotubes (on day 8 in differentiation medium) at a concentration of 5 μM for 24 hours; n=5 independent experiments for each condition. Wild type (WT) immortalized myotubes and untreated KO myotubes were used for comparison. The cells were homogenized on ice in deionized H_2O, sonicated, and centrifuged at 18,000×g at 4° ° C. for 15 min. The supernatants were used for measuring GAA activity and glycogen content.

[0291] Measurement of cell-associated GAA activity. The GAA activity in the cells was measured by using 4-methylumbelliferyl-α-D-glucopyranoside (4-MU-α-glucopyranoside; Sigma-Aldrich #M9766) as the fluorogenic GAA substrate as described.[13] Briefly, myotubes grown on Matrigel-coated 6-well plates were rinsed 3 times with PBS, homogenized in distilled water (using a syringe-based homogenization), sonicated, and centrifuged at 18,000×g at 4° C. for 15 min. The supernatants were incubated with the substrate in 0.2 M sodium acetate buffer (pH 4.3) in 96-well plates for 1 h at 37° C.; the reaction was stopped by adding 0.5 M carbonate buffer (pH 10.5). 4-Methylumbelliferone (4-MU; Sigma-Aldrich #M1381) was used as a standard. Fluorescence was measured on a multi-label plate reader (TECAN, SPARK 10M) at 360 nm excitation/465 nm emission.

[0292] Measurement of the glycogen content. The glycogen content was measured as the amount of glucose released after glycogen digestion with *Aspergillus Niger* amyloglucosidase (Sigma-Aldrich). Samples were denatured at 100° C. for 3 min to inactivate endogenous enzymes, centrifuged at 9,000 RPM at room temperature for 3 min, and the supernatants were incubated with/or without 0.175 U/mL amyloglucosidase for 90 min at 37° C. in 0.1 M potassium acetate buffer (pH 5.5) and boiled again to stop the reaction. The released glucose was measured using Glucose (Hexokinase) Liquid Reagents (Fisher) as recommended by the manufacturer; the absorbance at 340 nm was read on the Agilent Technologies Cary 60 UV-VIS Spectrophotometer. Protein concentration (BCA assay) was measured and used to normalize the data.

[0293] Western blotting. The myotubes were extensively washed, homogenized in RIPA buffer (PBS containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease/phosphatase inhibitor cocktail), and centrifuged for 10 min at 18,000×g at 4° C. Protein concentrations of the supernatants were measured using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Inc.), and equal amounts of protein were run on SDS-PAGE gels (Invitrogen, Carlsbad, CA). Separated proteins were electro-transferred onto nitrocellulose membranes (Invitrogen, Carlsbad, CA, USA). Membranes were then treated with blocking buffer (5% nonfat milk), incubated with primary antibodies [rat monoclonal anti-mouse LAMP-1 (Lysosomal-Associated Membrane Protein 1; CD107a #553792) and mouse monoclonal anti-Rab5 (#610724) from BD Pharmingen; rabbit monoclonal anti-human GAA(EPR4716(2) from Abcam] overnight at 4ºC, washed, incubated with the appropriate Alexa Fluor-conjugated secondary antibodies and washed again. Horseradish peroxidase (HRP)-chemiluminescence was developed using Azure Radiance plus kit and scanned on imager (Azure Biosystems). Mouse monoclonal anti-GAPDH antibody (Abcam, ab9484) served as loading controls.

[0294] For immunofluorescence, cultured myotubes were fixed with 2% paraformaldehyde (PFA) for 30 min at room temperature, followed by several washes with PBS and incubation with blocking reagent (MOM kit; Vector Laboratories, Burlingame, CA) for 1 h at room temperature. Myotubes were then incubated with primary antibodies overnight at 4° C., washed with PBS, incubated with secondary antibodies for 2 h, and washed again before examination by confocal microscopy (Zeiss LSM 880).

[0295] Statistical significance was determined by one way ANOVA testing using Prism software. Error bars represent SD. * $P<0.05$ was considered statistically significant. ** indicate P-values<0.01; *** indicate P-values<0.001.

[0296] Each publication, patent, and patent publication cited in this disclosure is incorporated in reference herein in its entirety. The present disclosure is not intended to be limited only to the foregoing examples, but encompasses all such modifications and variations as come within the scope of the appended claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 27

<210> SEQ ID NO 1
<211> LENGTH: 621
<212> TYPE: PRT
<213> ORGANISM: Arthrobacter protophormiae

<400> SEQUENCE: 1

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Ser Thr Tyr Asn Gly Pro Leu Ser Ser His Trp Phe Pro Glu Glu Leu
1               5                  10                  15

Ala Gln Trp Glu Pro Asp Ser Asp Pro Asp Ala Pro Phe Asn Arg Ser
            20                  25                  30

His Val Pro Leu Glu Pro Gly Arg Val Ala Asn Arg Val Asn Ala Asn
        35                  40                  45

Ala Asp Lys Asp Ala His Leu Val Ser Leu Ser Ala Leu Asn Arg His
    50                  55                  60

Thr Ser Gly Val Pro Ser Gln Gly Ala Pro Val Phe Tyr Glu Asn Thr
65                  70                  75                  80

Phe Ser Tyr Trp His Tyr Thr Asp Leu Met Val Tyr Trp Ala Gly Ser
                85                  90                  95

Ala Gly Glu Gly Ile Ile Val Pro Pro Ser Ala Asp Val Ile Asp Ala
            100                 105                 110

Ser His Arg Asn Gly Val Pro Ile Leu Gly Asn Val Phe Phe Pro Pro
        115                 120                 125

Thr Val Tyr Gly Gly Gln Leu Glu Trp Leu Glu Gln Met Leu Glu Gln
    130                 135                 140

Glu Glu Asp Gly Ser Phe Pro Leu Ala Asp Lys Leu Leu Glu Val Ala
145                 150                 155                 160

Asp Tyr Tyr Gly Phe Asp Gly Trp Phe Ile Asn Gln Glu Thr Glu Gly
                165                 170                 175

Ala Asp Glu Gly Thr Ala Glu Ala Met Gln Ala Phe Leu Val Tyr Leu
            180                 185                 190

Gln Glu Gln Lys Pro Glu Gly Met His Ile Met Trp Tyr Asp Ser Met
        195                 200                 205

Ile Asp Thr Gly Ala Ile Ala Trp Gln Asn His Leu Thr Asp Arg Asn
    210                 215                 220

Lys Met Tyr Leu Gln Asn Gly Ser Thr Arg Val Ala Asp Ser Met Phe
225                 230                 235                 240

Leu Asn Phe Trp Trp Arg Asp Gln Arg Gln Ser Asn Glu Leu Ala Gln
                245                 250                 255

Ala Leu Gly Arg Ser Pro Tyr Asp Leu Tyr Ala Gly Val Asp Val Glu
            260                 265                 270

Ala Arg Gly Thr Ser Thr Pro Val Gln Trp Glu Gly Leu Phe Pro Glu
        275                 280                 285

Gly Glu Lys Ala His Thr Ser Leu Gly Leu Tyr Arg Pro Asp Trp Ala
    290                 295                 300

Phe Gln Ser Ser Glu Thr Met Glu Ala Phe Tyr Glu Lys Glu Leu Gln
305                 310                 315                 320

Phe Trp Val Gly Ser Thr Gly Asn Pro Ala Glu Thr Asp Gly Gln Ser
                325                 330                 335

Asn Trp Pro Gly Met Ala His Trp Phe Pro Ala Lys Ser Thr Ala Thr
            340                 345                 350
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Ser Val Pro Phe Val Thr His Phe Asn Thr Gly Ser Gly Ala Gln Phe
        355                 360                 365

Ser Ala Glu Gly Lys Thr Val Ser Glu Gln Glu Trp Asn Asn Arg Ser
    370                 375                 380

Leu Gln Asp Val Leu Pro Thr Trp Arg Trp Ile Gln His Gly Gly Asp
385                 390                 395                 400

Leu Glu Ala Thr Phe Ser Trp Glu Glu Ala Phe Glu Gly Gly Ser Ser
                405                 410                 415

Leu Gln Trp His Gly Ser Leu Ala Glu Gly Glu His Ala Gln Ile Glu
            420                 425                 430

Leu Tyr Gln Thr Glu Leu Pro Ile Ser Glu Gly Thr Ser Leu Thr Trp
        435                 440                 445

Thr Phe Lys Ser Glu His Gly Asn Asp Leu Asn Val Gly Phe Arg Leu
    450                 455                 460

Asp Gly Glu Glu Asp Phe Arg Tyr Val Glu Gly Glu Gln Arg Glu Ser
465                 470                 475                 480

Ile Asn Gly Trp Thr Gln Trp Thr Leu Pro Leu Asp Ala Phe Ala Gly
                485                 490                 495

Gln Thr Ile Thr Gly Leu Ala Phe Ala Ala Glu Gly Asn Glu Thr Gly
            500                 505                 510

Leu Ala Glu Phe Tyr Ile Gly Gln Leu Ala Val Gly Ala Asp Ser Glu
        515                 520                 525

Lys Pro Ala Ala Pro Asn Val Asn Val Arg Gln Tyr Asp Pro Asp Pro
    530                 535                 540

Ser Gly Ile Gln Leu Val Trp Glu Lys Gln Ser Asn Val His His Tyr
545                 550                 555                 560

Arg Val Tyr Lys Glu Thr Lys His Gly Lys Glu Leu Ile Gly Thr Ser
                565                 570                 575

Ala Gly Asp Arg Ile Tyr Leu Glu Gly Leu Val Glu Glu Ser Lys Gln
            580                 585                 590

Asn Asp Val Arg Leu His Ile Glu Ala Leu Ser Glu Thr Phe Val Pro
        595                 600                 605

Ser Asp Ala Arg Met Ile Asp Ile Lys Ser Gly Ser Phe
    610                 615                 620
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<210> SEQ ID NO 2
<211> LENGTH: 290
<212> TYPE: PRT
<213> ORGANISM: Elizabethkingia meningoseptica

<400> SEQUENCE: 2

Thr Ala Leu Ala Gly Ser Asn Gly Val Cys Ile Ala Tyr Tyr Ile Thr
1               5                   10                  15

Asp Gly Arg Asn Ala Pro Thr Phe Lys Leu Lys Asp Ile Pro Asp Lys
            20                  25                  30

Val Asp Met Val Ile Leu Phe Gly Leu Lys Tyr Trp Ser Leu Gln Asp
        35                  40                  45

Thr Thr Lys Leu Pro Gly Gly Thr Gly Met Met Gly Ser Phe Lys Ser
    50                  55                  60

Tyr Lys Asp Leu Asp Thr Gln Ile Arg Ser Leu Gln Ser Arg Gly Ile
65                  70                  75                  80

Lys Val Leu Gln Asn Ile Asp Asp Asp Val Ser Trp Gln Ser Ser Lys
                85                  90                  95
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Pro Gly Gly Phe Ala Ser Ala Ala Ala Tyr Gly Asp Ala Ile Lys Ser
            100                 105                 110

Ile Val Ile Asp Lys Trp Lys Leu Asp Gly Ile Ser Leu Asp Ile Glu
        115                 120                 125

His Ser Gly Ala Lys Pro Asn Pro Ile Pro Thr Phe Pro Gly Tyr Ala
    130                 135                 140

Ala Thr Gly Tyr Asn Gly Trp Tyr Ser Gly Ser Met Ala Ala Thr Pro
145                 150                 155                 160

Ala Phe Leu Asn Val Ile Ser Glu Leu Thr Lys Tyr Phe Gly Thr Thr
                165                 170                 175

Ala Pro Asn Asn Lys Gln Leu Gln Ile Ala Ser Gly Ile Asp Val Tyr
            180                 185                 190

Ala Trp Asn Lys Ile Met Glu Asn Phe Arg Asn Asn Phe Asn Tyr Ile
        195                 200                 205

Gln Leu Gln Ser Tyr Gly Ala Asn Val Ser Arg Thr Gln Leu Met Met
    210                 215                 220

Asn Tyr Ala Thr Gly Thr Asn Lys Ile Pro Ala Ser Lys Met Val Phe
225                 230                 235                 240

Gly Ala Tyr Ala Glu Gly Gly Thr Asn Gln Ala Asn Asp Val Glu Val
                245                 250                 255

Ala Lys Trp Thr Pro Thr Gln Gly Ala Lys Gly Gly Met Met Ile Tyr
            260                 265                 270

Thr Tyr Asn Ser Asn Val Ser Tyr Ala Asn Ala Val Arg Asp Ala Val
        275                 280                 285

Lys Asn
    290
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<210> SEQ ID NO 3
<211> LENGTH: 787
<212> TYPE: PRT
<213> ORGANISM: Coprinus cinereus

<400> SEQUENCE: 3

```
Met Pro Ile Ala Gly Lys Lys Phe His Pro Arg Ala Leu Pro Glu Phe
1               5                   10                  15

Trp Arg Thr Phe Arg Glu Met Asp Glu Trp Arg Ala Thr Gln Thr Gly
            20                  25                  30

Pro Gln Ala Arg Pro Ala Glu Gly Ile Leu Lys Tyr Val Pro Arg Lys
        35                  40                  45

Ile Arg Pro Ala Asp Ile Ala Gly Lys Gly Arg Leu Leu Val Ser His
    50                  55                  60

Asp Tyr Lys Gly Gly Tyr Val Glu Asp Pro Phe Ser Lys Ser Tyr Ser
65                  70                  75                  80

Phe Asn Trp Trp Phe Ser Thr Asp Ser Phe Asn Tyr Phe Ala His His
                85                  90                  95

Arg Ile Thr Ile Pro Pro Pro Glu Trp Ile Asn Ala Ala His Arg Gln
            100                 105                 110

Gly Val Pro Ile Leu Gly Thr Ile Ile Phe Glu Gly Gly Ser Asp Glu
        115                 120                 125

Asp Ile Leu Arg Met Val Ile Gly Lys Thr Pro Gly Ser Thr Ser Asn
    130                 135                 140

Phe His Ala Glu Arg Asn Ala Glu Tyr Thr Val Pro Val Ser Ser Tyr
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145                 150                 155                 160

Tyr Ala Glu Leu Phe Ala Asp Leu Ala Val Glu Arg Gly Phe Asp Gly
                165                 170                 175

Trp Leu Leu Asn Val Glu Ile Gly Leu Gln Gly Gly Ser Glu Gln Ala
            180                 185                 190

Arg Gly Leu Ala Ala Trp Val Ala Leu Leu Gln Gln Glu Val Leu Lys
        195                 200                 205

Lys Val Gly Pro His Gly Leu Val Ile Trp Tyr Asp Ser Val Thr Val
    210                 215                 220

Arg Gly Asp Leu Trp Trp Gln Asp Arg Leu Asn Ala Phe Asn Leu Pro
225                 230                 235                 240

Phe Phe Leu Asn Ser Ser Gly Ile Phe Thr Asn Tyr Trp Trp Tyr Asn
                245                 250                 255

Asp Ala Pro Gln Lys Gln Ile Asp Phe Leu Ser Arg Val Asp Pro Asn
            260                 265                 270

Leu Thr Gly Gln Thr Ala Glu Pro His Gln Tyr Asn Leu Gln Lys Thr
        275                 280                 285

Ile Gln Asp Ile Tyr Ile Gly Val Asp Val Trp Gly Arg Gly Ser His
    290                 295                 300

Gly Gly Gly Gly Phe Gly Ala Tyr Lys Ala Ile Glu His Ala Asp Pro
305                 310                 315                 320

Lys Gly Leu Gly Phe Ser Val Ala Leu Phe Ala Gln Gly Trp Thr Trp
                325                 330                 335

Glu Thr Glu Glu Glu Lys Pro Gly Trp Asn Trp Ala Gln Phe Trp Asp
            340                 345                 350

Tyr Asp Ser Lys Leu Trp Val Gly Pro Pro Gly Val Val Glu Ala Pro
        355                 360                 365

Asp His Thr Val Lys Pro Gly Glu Tyr Pro Cys Val His Gly Pro Phe
    370                 375                 380

Gln Pro Ile Ser Ser Phe Phe Leu Thr Tyr Pro Pro Pro Asp Pro Leu
385                 390                 395                 400

Asp Leu Pro Phe Tyr Thr Asn Phe Cys Pro Gly Ile Gly Asp Ala Trp
                405                 410                 415

Phe Val Glu Gly Lys Glu Val Phe Arg Ser Glu Thr Gly Trp Thr Asp
            420                 425                 430

Met Asp Lys Gln Thr Thr Val Gly Asp Leu Val Trp Pro Arg Pro Lys
        435                 440                 445

Ile Tyr Asp Leu Pro Ser Gln Asn Ala Ser Gln Ala Thr Leu Asn Ala
    450                 455                 460

Ala Phe Asn Phe Asn Asp Ala Trp Asn Gly Gly Asn Ser Leu Gln Ile
465                 470                 475                 480

Asn Leu Thr Val Pro Gly Gly Ala Thr Thr Tyr Gly Ala Tyr Trp Val
                485                 490                 495

Pro Ile Gln Thr Phe Thr Phe Ser Ser Arg Arg Gln Tyr Glu Ala Ser
            500                 505                 510

Ile Val Tyr Lys Pro Gly Leu Ser Gly Lys Thr Arg Phe Asp Ala Lys
        515                 520                 525

Tyr Glu Val Gly Ile Arg Thr Ile Thr Gly Glu Asp Gln Gly Lys Ile
    530                 535                 540

Ile Ser Asn Thr Thr Thr Glu Val Gly Asn Gly Trp Arg Lys Val His
545                 550                 555                 560
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Ile Leu Phe Glu Ile Glu Thr Pro Val Glu Gly Gly Ser Ile Ile Val
               565                 570                 575

Pro Ser Ser Ile Gly Leu Val Ile Ala Val Ser Asn Val Ser Thr Thr
            580                 585                 590

Glu Gln Phe Glu Phe Pro Phe Leu Val Gly Gln Ile Thr Ile His Pro
        595                 600                 605

His Leu Pro Asp Arg Tyr Lys Glu Phe Lys Pro Ala Leu Leu Trp Leu
    610                 615                 620

Leu Phe Thr Pro Ser Ala Gly Thr Asn Ser Leu Asp Gly Thr Leu Thr
625                 630                 635                 640

Trp Asp Val Val Ala Ala Ile Glu Arg Pro Pro Pro Val Glu Ile Asn
                645                 650                 655

Asn Pro Asp Asp Ala Gln Ile Pro Trp Asn Leu Gln Pro Thr Lys Gln
            660                 665                 670

Glu Trp Phe Pro Asp Phe Leu Tyr Phe Asn Val Tyr Val Leu Glu Leu
        675                 680                 685

Leu Asp Gly Gly Gly Gln Gly Pro Pro Gln Trp Ile Gly Thr Thr Gly
    690                 695                 700

Tyr Asp Gly Glu Lys Lys Arg Phe Phe Ile Tyr Asp Glu Ser Leu Pro
705                 710                 715                 720

Pro Thr Ser Gly Leu Arg Arg Phe Thr Phe Gln Ile Glu Gly Val Leu
                725                 730                 735

Glu Thr Gly Glu Ser Thr His Trp Tyr Asp Ala Pro Ala Ala Pro Ser
            740                 745                 750

Ala Thr Ala Gly Gly Glu Gln Lys Arg Thr Arg Arg Thr Ser Leu Lys
        755                 760                 765

Ser Val Leu Ser Pro Leu Arg Arg Lys Lys Ser Lys Gly Asp Ile Ser
    770                 775                 780

Val Ala Lys
785


<210> SEQ ID NO 4
<211> LENGTH: 429
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Gln Leu Arg Asn Pro Glu Leu His Leu Gly Cys Ala Leu Ala Leu
1               5                   10                  15

Arg Phe Leu Ala Leu Val Ser Trp Asp Ile Pro Gly Ala Arg Ala Leu
            20                  25                  30

Asp Asn Gly Leu Ala Arg Thr Pro Thr Met Gly Trp Leu His Trp Glu
        35                  40                  45

Arg Phe Met Cys Asn Leu Asp Cys Gln Glu Glu Pro Asp Ser Cys Ile
    50                  55                  60

Ser Glu Lys Leu Phe Met Glu Met Ala Glu Leu Met Val Ser Glu Gly
65                  70                  75                  80

Trp Lys Asp Ala Gly Tyr Glu Tyr Leu Cys Ile Asp Asp Cys Trp Met
                85                  90                  95

Ala Pro Gln Arg Asp Ser Glu Gly Arg Leu Gln Ala Asp Pro Gln Arg
            100                 105                 110

Phe Pro His Gly Ile Arg Gln Leu Ala Asn Tyr Val His Ser Lys Gly
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        115                 120                 125

Leu Lys Leu Gly Ile Tyr Ala Asp Val Gly Asn Lys Thr Cys Ala Gly
    130                 135                 140

Phe Pro Gly Ser Phe Gly Tyr Tyr Asp Ile Asp Ala Gln Thr Phe Ala
145                 150                 155                 160

Asp Trp Gly Val Asp Leu Leu Lys Phe Asp Gly Cys Tyr Cys Asp Ser
                165                 170                 175

Leu Glu Asn Leu Ala Asp Gly Tyr Lys His Met Ser Leu Ala Leu Asn
            180                 185                 190

Arg Thr Gly Arg Ser Ile Val Tyr Ser Cys Glu Trp Pro Leu Tyr Met
        195                 200                 205

Trp Pro Phe Gln Lys Pro Asn Tyr Thr Glu Ile Arg Gln Tyr Cys Asn
    210                 215                 220

His Trp Arg Asn Phe Ala Asp Ile Asp Asp Ser Trp Lys Ser Ile Lys
225                 230                 235                 240

Ser Ile Leu Asp Trp Thr Ser Phe Asn Gln Glu Arg Ile Val Asp Val
                245                 250                 255

Ala Gly Pro Gly Gly Trp Asn Asp Pro Asp Met Leu Val Ile Gly Asn
            260                 265                 270

Phe Gly Leu Ser Trp Asn Gln Gln Val Thr Gln Met Ala Leu Trp Ala
        275                 280                 285

Ile Met Ala Ala Pro Leu Phe Met Ser Asn Asp Leu Arg His Ile Ser
    290                 295                 300

Pro Gln Ala Lys Ala Leu Leu Gln Asp Lys Asp Val Ile Ala Ile Asn
305                 310                 315                 320

Gln Asp Pro Leu Gly Lys Gln Gly Tyr Gln Leu Arg Gln Gly Asp Asn
                325                 330                 335

Phe Glu Val Trp Glu Arg Pro Leu Ser Gly Leu Ala Trp Ala Val Ala
            340                 345                 350

Met Ile Asn Arg Gln Glu Ile Gly Gly Pro Arg Ser Tyr Thr Ile Ala
        355                 360                 365

Val Ala Ser Leu Gly Lys Gly Val Ala Cys Asn Pro Ala Cys Phe Ile
    370                 375                 380

Thr Gln Leu Leu Pro Val Lys Arg Lys Leu Gly Phe Tyr Glu Trp Thr
385                 390                 395                 400

Ser Arg Leu Arg Ser His Ile Asn Pro Thr Gly Thr Val Leu Leu Gln
                405                 410                 415

Leu Glu Asn Thr Met Gln Met Ser Leu Lys Asp Leu Leu
            420                 425


<210> SEQ ID NO 5
<211> LENGTH: 395
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Met Pro Gly Arg Ser Cys Val Ala Leu Val Leu Leu Ala Ala Ala Val
1               5                   10                  15

Ser Cys Ala Val Ala Gln His Ala Pro Pro Trp Thr Glu Asp Cys Arg
            20                  25                  30

Lys Ser Thr Tyr Pro Pro Ser Gly Pro Thr Tyr Arg Gly Ala Val Pro
        35                  40                  45
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Trp Tyr Thr Ile Asn Leu Asp Leu Pro Pro Tyr Lys Arg Trp His Glu
    50                  55                  60

Leu Met Leu Asp Lys Ala Pro Val Leu Lys Val Ile Val Asn Ser Leu
65                  70                  75                  80

Lys Asn Met Ile Asn Thr Phe Val Pro Ser Gly Lys Ile Met Gln Val
                85                  90                  95

Val Asp Glu Lys Leu Pro Gly Leu Leu Gly Asn Phe Pro Gly Pro Phe
            100                 105                 110

Glu Glu Glu Met Lys Gly Ile Ala Ala Val Thr Asp Ile Pro Leu Gly
        115                 120                 125

Glu Ile Ile Ser Phe Asn Ile Phe Tyr Glu Leu Phe Thr Ile Cys Thr
    130                 135                 140

Ser Ile Val Ala Glu Asp Lys Lys Gly His Leu Ile His Gly Arg Asn
145                 150                 155                 160

Met Asp Phe Gly Val Phe Leu Gly Trp Asn Ile Asn Asn Asp Thr Trp
                165                 170                 175

Val Ile Thr Glu Gln Leu Lys Pro Leu Thr Val Asn Leu Asp Phe Gln
            180                 185                 190

Arg Asn Asn Lys Thr Val Phe Lys Ala Ser Ser Phe Ala Gly Tyr Val
        195                 200                 205

Gly Met Leu Thr Gly Phe Lys Pro Gly Leu Phe Ser Leu Thr Leu Asn
    210                 215                 220

Glu Arg Phe Ser Ile Asn Gly Gly Tyr Leu Gly Ile Leu Glu Trp Ile
225                 230                 235                 240

Leu Gly Lys Lys Asp Val Met Trp Ile Gly Phe Leu Thr Arg Thr Val
                245                 250                 255

Leu Glu Asn Ser Thr Ser Tyr Glu Glu Ala Lys Asn Leu Leu Thr Lys
            260                 265                 270

Thr Lys Ile Leu Ala Pro Ala Tyr Phe Ile Leu Gly Gly Asn Gln Ser
        275                 280                 285

Gly Glu Gly Cys Val Ile Thr Arg Asp Arg Lys Glu Ser Leu Asp Val
    290                 295                 300

Tyr Glu Leu Asp Ala Lys Gln Gly Arg Trp Tyr Val Val Gln Thr Asn
305                 310                 315                 320

Tyr Asp Arg Trp Lys His Pro Phe Phe Leu Asp Asp Arg Arg Thr Pro
                325                 330                 335

Ala Lys Met Cys Leu Asn Arg Thr Ser Gln Glu Asn Ile Ser Phe Glu
            340                 345                 350

Thr Met Tyr Asp Val Leu Ser Thr Lys Pro Val Leu Asn Lys Leu Thr
        355                 360                 365

Val Tyr Thr Thr Leu Ile Asp Val Thr Lys Gly Gln Phe Glu Thr Tyr
    370                 375                 380

Leu Arg Asp Cys Pro Asp Pro Cys Ile Gly Trp
385                 390                 395


<210> SEQ ID NO 6
<211> LENGTH: 466
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Arg Ala Pro Gly Met Arg Ser Arg Pro Ala Gly Pro Ala Leu Leu
1               5                   10                  15
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Leu Leu Leu Leu Phe Leu Gly Ala Ala Glu Ser Val Arg Arg Ala Gln
            20                  25                  30

Pro Pro Arg Arg Tyr Thr Pro Asp Trp Pro Ser Leu Asp Ser Arg Pro
        35                  40                  45

Leu Pro Ala Trp Phe Asp Glu Ala Lys Phe Gly Val Phe Ile His Trp
    50                  55                  60

Gly Val Phe Ser Val Pro Ala Trp Gly Ser Glu Trp Phe Trp Trp His
65                  70                  75                  80

Trp Gln Gly Glu Gly Arg Pro Gln Tyr Gln Arg Phe Met Arg Asp Asn
                85                  90                  95

Tyr Pro Pro Gly Phe Ser Tyr Ala Asp Phe Gly Pro Gln Phe Thr Ala
            100                 105                 110

Arg Phe Phe His Pro Glu Glu Trp Ala Asp Leu Phe Gln Ala Ala Gly
        115                 120                 125

Ala Lys Tyr Val Val Leu Thr Thr Lys His His Glu Gly Phe Thr Asn
    130                 135                 140

Trp Pro Ser Pro Val Ser Trp Asn Trp Asn Ser Lys Asp Val Gly Pro
145                 150                 155                 160

His Arg Asp Leu Val Gly Glu Leu Gly Thr Ala Leu Arg Lys Arg Asn
                165                 170                 175

Ile Arg Tyr Gly Leu Tyr His Ser Leu Leu Glu Trp Phe His Pro Leu
            180                 185                 190

Tyr Leu Leu Asp Lys Lys Asn Gly Phe Lys Thr Gln His Phe Val Ser
        195                 200                 205

Ala Lys Thr Met Pro Glu Leu Tyr Asp Leu Val Asn Ser Tyr Lys Pro
    210                 215                 220

Asp Leu Ile Trp Ser Asp Gly Glu Trp Glu Cys Pro Asp Thr Tyr Trp
225                 230                 235                 240

Asn Ser Thr Asn Phe Leu Ser Trp Leu Tyr Asn Asp Ser Pro Val Lys
                245                 250                 255

Asp Glu Val Val Val Asn Asp Arg Trp Gly Gln Asn Cys Ser Cys His
            260                 265                 270

His Gly Gly Tyr Tyr Asn Cys Glu Asp Lys Phe Lys Pro Gln Ser Leu
        275                 280                 285

Pro Asp His Lys Trp Glu Met Cys Thr Ser Ile Asp Lys Phe Ser Trp
    290                 295                 300

Gly Tyr Arg Arg Asp Met Ala Leu Ser Asp Val Thr Glu Glu Ser Glu
305                 310                 315                 320

Ile Ile Ser Glu Leu Val Gln Thr Val Ser Leu Gly Gly Asn Tyr Leu
                325                 330                 335

Leu Asn Ile Gly Pro Thr Lys Asp Gly Leu Ile Val Pro Ile Phe Gln
            340                 345                 350

Glu Arg Leu Leu Ala Val Gly Lys Trp Leu Ser Ile Asn Gly Glu Ala
        355                 360                 365

Ile Tyr Ala Ser Lys Pro Trp Arg Val Gln Trp Glu Lys Asn Thr Thr
    370                 375                 380

Ser Val Trp Tyr Thr Ser Lys Gly Ser Ala Val Tyr Ala Ile Phe Leu
385                 390                 395                 400

His Trp Pro Glu Asn Gly Val Leu Asn Leu Glu Ser Pro Ile Thr Thr
                405                 410                 415
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Ser Thr Thr Lys Ile Thr Met Leu Gly Ile Gln Gly Asp Leu Lys Trp
            420                 425                 430

Ser Thr Asp Pro Asp Lys Gly Leu Phe Ile Ser Leu Pro Gln Leu Pro
        435                 440                 445

Pro Ser Ala Val Pro Ala Glu Phe Ala Trp Thr Ile Lys Leu Thr Gly
    450                 455                 460

Val Lys
465
```

<210> SEQ ID NO 7
<211> LENGTH: 536
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

```
Met Glu Phe Ser Ser Pro Ser Arg Glu Glu Cys Pro Lys Pro Leu Ser
1               5                   10                  15

Arg Val Ser Ile Met Ala Gly Ser Leu Thr Gly Leu Leu Leu Leu Gln
            20                  25                  30

Ala Val Ser Trp Ala Ser Gly Ala Arg Pro Cys Ile Pro Lys Ser Phe
        35                  40                  45

Gly Tyr Ser Ser Val Val Cys Val Cys Asn Ala Thr Tyr Cys Asp Ser
    50                  55                  60

Phe Asp Pro Pro Thr Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu
65                  70                  75                  80

Ser Thr Arg Ser Gly Arg Arg Met Glu Leu Ser Met Gly Pro Ile Gln
                85                  90                  95

Ala Asn His Thr Gly Thr Gly Leu Leu Leu Thr Leu Gln Pro Glu Gln
            100                 105                 110

Lys Phe Gln Lys Val Lys Gly Phe Gly Gly Ala Met Thr Asp Ala Ala
        115                 120                 125

Ala Leu Asn Ile Leu Ala Leu Ser Pro Pro Ala Gln Asn Leu Leu Leu
    130                 135                 140

Lys Ser Tyr Phe Ser Glu Glu Gly Ile Gly Tyr Asn Ile Ile Arg Val
145                 150                 155                 160

Pro Met Ala Ser Cys Asp Phe Ser Ile Arg Thr Tyr Thr Tyr Ala Asp
                165                 170                 175

Thr Pro Asp Asp Phe Gln Leu His Asn Phe Ser Leu Pro Glu Glu Asp
            180                 185                 190

Thr Lys Leu Lys Ile Pro Leu Ile His Arg Ala Leu Gln Leu Ala Gln
        195                 200                 205

Arg Pro Val Ser Leu Leu Ala Ser Pro Trp Thr Ser Pro Thr Trp Leu
    210                 215                 220

Lys Thr Asn Gly Ala Val Asn Gly Lys Gly Ser Leu Lys Gly Gln Pro
225                 230                 235                 240

Gly Asp Ile Tyr His Gln Thr Trp Ala Arg Tyr Phe Val Lys Phe Leu
                245                 250                 255

Asp Ala Tyr Ala Glu His Lys Leu Gln Phe Trp Ala Val Thr Ala Glu
            260                 265                 270

Asn Glu Pro Ser Ala Gly Leu Leu Ser Gly Tyr Pro Phe Gln Cys Leu
        275                 280                 285

Gly Phe Thr Pro Glu His Gln Arg Asp Phe Ile Ala Arg Asp Leu Gly
    290                 295                 300
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```
Pro Thr Leu Ala Asn Ser Thr His His Asn Val Arg Leu Leu Met Leu
305                 310                 315                 320

Asp Asp Gln Arg Leu Leu Leu Pro His Trp Ala Lys Val Val Leu Thr
                325                 330                 335

Asp Pro Glu Ala Ala Lys Tyr Val His Gly Ile Ala Val His Trp Tyr
            340                 345                 350

Leu Asp Phe Leu Ala Pro Ala Lys Ala Thr Leu Gly Glu Thr His Arg
        355                 360                 365

Leu Phe Pro Asn Thr Met Leu Phe Ala Ser Glu Ala Cys Val Gly Ser
    370                 375                 380

Lys Phe Trp Glu Gln Ser Val Arg Leu Gly Ser Trp Asp Arg Gly Met
385                 390                 395                 400

Gln Tyr Ser His Ser Ile Ile Thr Asn Leu Leu Tyr His Val Val Gly
                405                 410                 415

Trp Thr Asp Trp Asn Leu Ala Leu Asn Pro Glu Gly Gly Pro Asn Trp
            420                 425                 430

Val Arg Asn Phe Val Asp Ser Pro Ile Ile Val Asp Ile Thr Lys Asp
        435                 440                 445

Thr Phe Tyr Lys Gln Pro Met Phe Tyr His Leu Gly His Phe Ser Lys
    450                 455                 460

Phe Ile Pro Glu Gly Ser Gln Arg Val Gly Leu Val Ala Ser Gln Lys
465                 470                 475                 480

Asn Asp Leu Asp Ala Val Ala Leu Met His Pro Asp Gly Ser Ala Val
                485                 490                 495

Val Val Val Leu Asn Arg Ser Ser Lys Asp Val Pro Leu Thr Ile Lys
            500                 505                 510

Asp Pro Ala Val Gly Phe Leu Glu Thr Ile Ser Pro Gly Tyr Ser Ile
        515                 520                 525

His Thr Tyr Leu Trp Arg Arg Gln
    530                 535


<210> SEQ ID NO 8
<211> LENGTH: 677
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Pro Gly Phe Leu Val Arg Ile Leu Pro Leu Leu Leu Val Leu Leu
1               5                   10                  15

Leu Leu Gly Pro Thr Arg Gly Leu Arg Asn Ala Thr Gln Arg Met Phe
            20                  25                  30

Glu Ile Asp Tyr Ser Arg Asp Ser Phe Leu Lys Asp Gly Gln Pro Phe
        35                  40                  45

Arg Tyr Ile Ser Gly Ser Ile His Tyr Ser Arg Val Pro Arg Phe Tyr
    50                  55                  60

Trp Lys Asp Arg Leu Leu Lys Met Lys Met Ala Gly Leu Asn Ala Ile
65                  70                  75                  80

Gln Thr Tyr Val Pro Trp Asn Phe His Glu Pro Trp Pro Gly Gln Tyr
                85                  90                  95

Gln Phe Ser Glu Asp His Asp Val Glu Tyr Phe Leu Arg Leu Ala His
            100                 105                 110

Glu Leu Gly Leu Leu Val Ile Leu Arg Pro Gly Pro Tyr Ile Cys Ala
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        115                 120                 125

Glu Trp Glu Met Gly Gly Leu Pro Ala Trp Leu Leu Glu Lys Glu Ser
    130                 135                 140

Ile Leu Leu Arg Ser Ser Asp Pro Asp Tyr Leu Ala Ala Val Asp Lys
145                 150                 155                 160

Trp Leu Gly Val Leu Leu Pro Lys Met Lys Pro Leu Leu Tyr Gln Asn
                165                 170                 175

Gly Gly Pro Val Ile Thr Val Gln Val Glu Asn Glu Tyr Gly Ser Tyr
            180                 185                 190

Phe Ala Cys Asp Phe Asp Tyr Leu Arg Phe Leu Gln Lys Arg Phe Arg
        195                 200                 205

His His Leu Gly Asp Asp Val Val Leu Phe Thr Thr Asp Gly Ala His
    210                 215                 220

Lys Thr Phe Leu Lys Cys Gly Ala Leu Gln Gly Leu Tyr Thr Thr Val
225                 230                 235                 240

Asp Phe Gly Thr Gly Ser Asn Ile Thr Asp Ala Phe Leu Ser Gln Arg
                245                 250                 255

Lys Cys Glu Pro Lys Gly Pro Leu Ile Asn Ser Glu Phe Tyr Thr Gly
            260                 265                 270

Trp Leu Asp His Trp Gly Gln Pro His Ser Thr Ile Lys Thr Glu Ala
        275                 280                 285

Val Ala Ser Ser Leu Tyr Asp Ile Leu Ala Arg Gly Ala Ser Val Asn
    290                 295                 300

Leu Tyr Met Phe Ile Gly Gly Thr Asn Phe Ala Tyr Trp Asn Gly Ala
305                 310                 315                 320

Asn Ser Pro Tyr Ala Ala Gln Pro Thr Ser Tyr Asp Tyr Asp Ala Pro
                325                 330                 335

Leu Ser Glu Ala Gly Asp Leu Thr Glu Lys Tyr Phe Ala Leu Arg Asn
            340                 345                 350

Ile Ile Gln Lys Phe Glu Lys Val Pro Glu Gly Pro Ile Pro Pro Ser
        355                 360                 365

Thr Pro Lys Phe Ala Tyr Gly Lys Val Thr Leu Glu Lys Leu Lys Thr
    370                 375                 380

Val Gly Ala Ala Leu Asp Ile Leu Cys Pro Ser Gly Pro Ile Lys Ser
385                 390                 395                 400

Leu Tyr Pro Leu Thr Phe Ile Gln Val Lys Gln His Tyr Gly Phe Val
                405                 410                 415

Leu Tyr Arg Thr Thr Leu Pro Gln Asp Cys Ser Asn Pro Ala Pro Leu
            420                 425                 430

Ser Ser Pro Leu Asn Gly Val His Asp Arg Ala Tyr Val Ala Val Asp
        435                 440                 445

Gly Ile Pro Gln Gly Val Leu Glu Arg Asn Asn Val Ile Thr Leu Asn
    450                 455                 460

Ile Thr Gly Lys Ala Gly Ala Thr Leu Asp Leu Leu Val Glu Asn Met
465                 470                 475                 480

Gly Arg Val Asn Tyr Gly Ala Tyr Ile Asn Asp Phe Lys Gly Leu Val
                485                 490                 495

Ser Asn Leu Thr Leu Ser Ser Asn Ile Leu Thr Asp Trp Thr Ile Phe
            500                 505                 510

Pro Leu Asp Thr Glu Asp Ala Val Arg Ser His Leu Gly Gly Trp Gly
        515                 520                 525
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His Arg Asp Ser Gly His His Asp Glu Ala Trp Ala His Asn Ser Ser
    530                 535                 540

Asn Tyr Thr Leu Pro Ala Phe Tyr Met Gly Asn Phe Ser Ile Pro Ser
545                 550                 555                 560

Gly Ile Pro Asp Leu Pro Gln Asp Thr Phe Ile Gln Phe Pro Gly Trp
                565                 570                 575

Thr Lys Gly Gln Val Trp Ile Asn Gly Phe Asn Leu Gly Arg Tyr Trp
            580                 585                 590

Pro Ala Arg Gly Pro Gln Leu Thr Leu Phe Val Pro Gln His Ile Leu
        595                 600                 605

Met Thr Ser Ala Pro Asn Thr Ile Thr Val Leu Glu Leu Glu Trp Ala
    610                 615                 620

Pro Cys Ser Ser Asp Asp Pro Glu Leu Cys Ala Val Thr Phe Val Asp
625                 630                 635                 640

Arg Pro Val Ile Gly Ser Ser Val Thr Tyr Asp His Pro Ser Lys Pro
                645                 650                 655

Val Glu Lys Arg Leu Met Pro Pro Pro Gln Lys Asn Lys Asp Ser
            660                 665                 670

Trp Leu Asp His Val
        675


<210> SEQ ID NO 9
<211> LENGTH: 550
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Met Pro Pro Pro Arg Thr Gly Arg Gly Leu Leu Trp Leu Gly Leu Val
1               5                   10                  15

Leu Ser Ser Val Cys Val Ala Leu Gly Ser Glu Thr Gln Ala Asn Ser
            20                  25                  30

Thr Thr Asp Ala Leu Asn Val Leu Leu Ile Ile Val Asp Asp Leu Arg
        35                  40                  45

Pro Ser Leu Gly Cys Tyr Gly Asp Lys Leu Val Arg Ser Pro Asn Ile
    50                  55                  60

Asp Gln Leu Ala Ser His Ser Leu Leu Phe Gln Asn Ala Phe Ala Gln
65                  70                  75                  80

Gln Ala Val Cys Ala Pro Ser Arg Val Ser Phe Leu Thr Gly Arg Arg
                85                  90                  95

Pro Asp Thr Thr Arg Leu Tyr Asp Phe Asn Ser Tyr Trp Arg Val His
            100                 105                 110

Ala Gly Asn Phe Ser Thr Ile Pro Gln Tyr Phe Lys Glu Asn Gly Tyr
        115                 120                 125

Val Thr Met Ser Val Gly Lys Val Phe His Pro Gly Ile Ser Ser Asn
    130                 135                 140

His Thr Asp Asp Ser Pro Tyr Ser Trp Ser Phe Pro Pro Tyr His Pro
145                 150                 155                 160

Ser Ser Glu Lys Tyr Glu Asn Thr Lys Thr Cys Arg Gly Pro Asp Gly
                165                 170                 175

Glu Leu His Ala Asn Leu Leu Cys Pro Val Asp Val Leu Asp Val Pro
            180                 185                 190

Glu Gly Thr Leu Pro Asp Lys Gln Ser Thr Glu Gln Ala Ile Gln Leu
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```
        195                 200                 205

Leu Glu Lys Met Lys Thr Ser Ala Ser Pro Phe Phe Leu Ala Val Gly
    210                 215                 220

Tyr His Lys Pro His Ile Pro Phe Arg Tyr Pro Lys Glu Phe Gln Lys
225                 230                 235                 240

Leu Tyr Pro Leu Glu Asn Ile Thr Leu Ala Pro Asp Pro Glu Val Pro
                245                 250                 255

Asp Gly Leu Pro Pro Val Ala Tyr Asn Pro Trp Met Asp Ile Arg Gln
            260                 265                 270

Arg Glu Asp Val Gln Ala Leu Asn Ile Ser Val Pro Tyr Gly Pro Ile
        275                 280                 285

Pro Val Asp Phe Gln Arg Lys Ile Arg Gln Ser Tyr Phe Ala Ser Val
    290                 295                 300

Ser Tyr Leu Asp Thr Gln Val Gly Arg Leu Leu Ser Ala Leu Asp Asp
305                 310                 315                 320

Leu Gln Leu Ala Asn Ser Thr Ile Ile Ala Phe Thr Ser Asp His Gly
                325                 330                 335

Trp Ala Leu Gly Glu His Gly Glu Trp Ala Lys Tyr Ser Asn Phe Asp
            340                 345                 350

Val Ala Thr His Val Pro Leu Ile Phe Tyr Val Pro Gly Arg Thr Ala
        355                 360                 365

Ser Leu Pro Glu Ala Gly Glu Lys Leu Phe Pro Tyr Leu Asp Pro Phe
    370                 375                 380

Asp Ser Ala Ser Gln Leu Met Glu Pro Gly Arg Gln Ser Met Asp Leu
385                 390                 395                 400

Val Glu Leu Val Ser Leu Phe Pro Thr Leu Ala Gly Leu Ala Gly Leu
                405                 410                 415

Gln Val Pro Pro Arg Cys Pro Val Pro Ser Phe His Val Glu Leu Cys
            420                 425                 430

Arg Glu Gly Lys Asn Leu Leu Lys His Phe Arg Phe Arg Asp Leu Glu
        435                 440                 445

Glu Asp Pro Tyr Leu Pro Gly Asn Pro Arg Glu Leu Ile Ala Tyr Ser
    450                 455                 460

Gln Tyr Pro Arg Pro Ser Asp Ile Pro Gln Trp Asn Ser Asp Lys Pro
465                 470                 475                 480

Ser Leu Lys Asp Ile Lys Ile Met Gly Tyr Ser Ile Arg Thr Ile Asp
                485                 490                 495

Tyr Arg Tyr Thr Val Trp Val Gly Phe Asn Pro Asp Glu Phe Leu Ala
            500                 505                 510

Asn Phe Ser Asp Ile His Ala Gly Glu Leu Tyr Phe Val Asp Ser Asp
        515                 520                 525

Pro Leu Gln Asp His Asn Met Tyr Asn Asp Ser Gln Gly Gly Asp Leu
    530                 535                 540

Phe Gln Leu Leu Met Pro
545                 550


<210> SEQ ID NO 10
<211> LENGTH: 653
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10
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```
Met Arg Pro Leu Arg Pro Arg Ala Ala Leu Leu Ala Leu Leu Ala Ser
1               5                  10                  15

Leu Leu Ala Ala Pro Pro Val Ala Pro Ala Glu Ala Pro His Leu Val
            20                  25                  30

His Val Asp Ala Ala Arg Ala Leu Trp Pro Leu Arg Arg Phe Trp Arg
        35                  40                  45

Ser Thr Gly Phe Cys Pro Pro Leu Pro His Ser Gln Ala Asp Gln Tyr
    50                  55                  60

Val Leu Ser Trp Asp Gln Gln Leu Asn Leu Ala Tyr Val Gly Ala Val
65                  70                  75                  80

Pro His Arg Gly Ile Lys Gln Val Arg Thr His Trp Leu Leu Glu Leu
                85                  90                  95

Val Thr Thr Arg Gly Ser Thr Gly Arg Gly Leu Ser Tyr Asn Phe Thr
            100                 105                 110

His Leu Asp Gly Tyr Leu Asp Leu Leu Arg Glu Asn Gln Leu Leu Pro
        115                 120                 125

Gly Phe Glu Leu Met Gly Ser Ala Ser Gly His Phe Thr Asp Phe Glu
    130                 135                 140

Asp Lys Gln Gln Val Phe Glu Trp Lys Asp Leu Val Ser Ser Leu Ala
145                 150                 155                 160

Arg Arg Tyr Ile Gly Arg Tyr Gly Leu Ala His Val Ser Lys Trp Asn
                165                 170                 175

Phe Glu Thr Trp Asn Glu Pro Asp His His Asp Phe Asp Asn Val Ser
            180                 185                 190

Met Thr Met Gln Gly Phe Leu Asn Tyr Tyr Asp Ala Cys Ser Glu Gly
        195                 200                 205

Leu Arg Ala Ala Ser Pro Ala Leu Arg Leu Gly Gly Pro Gly Asp Ser
    210                 215                 220

Phe His Thr Pro Pro Arg Ser Pro Leu Ser Trp Gly Leu Leu Arg His
225                 230                 235                 240

Cys His Asp Gly Thr Asn Phe Phe Thr Gly Glu Ala Gly Val Arg Leu
                245                 250                 255

Asp Tyr Ile Ser Leu His Arg Lys Gly Ala Arg Ser Ser Ile Ser Ile
            260                 265                 270

Leu Glu Gln Glu Lys Val Val Ala Gln Gln Ile Arg Gln Leu Phe Pro
        275                 280                 285

Lys Phe Ala Asp Thr Pro Ile Tyr Asn Asp Glu Ala Asp Pro Leu Val
    290                 295                 300

Gly Trp Ser Leu Pro Gln Pro Trp Arg Ala Asp Val Thr Tyr Ala Ala
305                 310                 315                 320

Met Val Val Lys Val Ile Ala Gln His Gln Asn Leu Leu Leu Ala Asn
                325                 330                 335

Thr Thr Ser Ala Phe Pro Tyr Ala Leu Leu Ser Asn Asp Asn Ala Phe
            340                 345                 350

Leu Ser Tyr His Pro His Pro Phe Ala Gln Arg Thr Leu Thr Ala Arg
        355                 360                 365

Phe Gln Val Asn Asn Thr Arg Pro Pro His Val Gln Leu Leu Arg Lys
    370                 375                 380

Pro Val Leu Thr Ala Met Gly Leu Leu Ala Leu Leu Asp Glu Glu Gln
385                 390                 395                 400

Leu Trp Ala Glu Val Ser Gln Ala Gly Thr Val Leu Asp Ser Asn His
```

-continued

```
                405                 410                 415

Thr Val Gly Val Leu Ala Ser Ala His Arg Pro Gln Gly Pro Ala Asp
            420                 425                 430

Ala Trp Arg Ala Ala Val Leu Ile Tyr Ala Ser Asp Asp Thr Arg Ala
        435                 440                 445

His Pro Asn Arg Ser Val Ala Val Thr Leu Arg Leu Arg Gly Val Pro
    450                 455                 460

Pro Gly Pro Gly Leu Val Tyr Val Thr Arg Tyr Leu Asp Asn Gly Leu
465                 470                 475                 480

Cys Ser Pro Asp Gly Glu Trp Arg Arg Leu Gly Arg Pro Val Phe Pro
                485                 490                 495

Thr Ala Glu Gln Phe Arg Arg Met Arg Ala Ala Glu Asp Pro Val Ala
            500                 505                 510

Ala Ala Pro Arg Pro Leu Pro Ala Gly Gly Arg Leu Thr Leu Arg Pro
        515                 520                 525

Ala Leu Arg Leu Pro Ser Leu Leu Leu Val His Val Cys Ala Arg Pro
    530                 535                 540

Glu Lys Pro Pro Gly Gln Val Thr Arg Leu Arg Ala Leu Pro Leu Thr
545                 550                 555                 560

Gln Gly Gln Leu Val Leu Val Trp Ser Asp Glu His Val Gly Ser Lys
                565                 570                 575

Cys Leu Trp Thr Tyr Glu Ile Gln Phe Ser Gln Asp Gly Lys Ala Tyr
            580                 585                 590

Thr Pro Val Ser Arg Lys Pro Ser Thr Phe Asn Leu Phe Val Phe Ser
        595                 600                 605

Pro Asp Thr Gly Ala Val Ser Gly Ser Tyr Arg Val Arg Ala Leu Asp
    610                 615                 620

Tyr Trp Ala Arg Pro Gly Pro Phe Ser Asp Pro Val Pro Tyr Leu Glu
625                 630                 635                 640

Val Pro Val Pro Arg Gly Pro Pro Ser Pro Gly Asn Pro
                645                 650
```

<210> SEQ ID NO 11
<211> LENGTH: 685
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

```
Met Ala Glu Trp Leu Leu Ser Ala Ser Trp Gln Arg Arg Ala Lys Ala
1               5                   10                  15

Met Thr Ala Ala Ala Gly Ser Ala Gly Arg Ala Ala Val Pro Leu Leu
            20                  25                  30

Leu Cys Ala Leu Leu Ala Pro Gly Gly Ala Tyr Val Leu Asp Asp Ser
        35                  40                  45

Asp Gly Leu Gly Arg Glu Phe Asp Gly Ile Gly Ala Val Ser Gly Gly
    50                  55                  60

Gly Ala Thr Ser Arg Leu Leu Val Asn Tyr Pro Glu Pro Tyr Arg Ser
65                  70                  75                  80

Gln Ile Leu Asp Tyr Leu Phe Lys Pro Asn Phe Gly Ala Ser Leu His
                85                  90                  95

Ile Leu Lys Val Glu Ile Gly Gly Asp Gly Gln Thr Thr Asp Gly Thr
            100                 105                 110
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```
Glu Pro Ser His Met His Tyr Ala Leu Asp Glu Asn Tyr Phe Arg Gly
        115                 120                 125

Tyr Glu Trp Trp Leu Met Lys Glu Ala Lys Lys Arg Asn Pro Asn Ile
    130                 135                 140

Thr Leu Ile Gly Leu Pro Trp Ser Phe Pro Gly Trp Leu Gly Lys Gly
145                 150                 155                 160

Phe Asp Trp Pro Tyr Val Asn Leu Gln Leu Thr Ala Tyr Tyr Val Val
                165                 170                 175

Thr Trp Ile Val Gly Ala Lys Arg Tyr His Asp Leu Asp Ile Asp Tyr
            180                 185                 190

Ile Gly Ile Trp Asn Glu Arg Ser Tyr Asn Ala Asn Tyr Ile Lys Ile
        195                 200                 205

Leu Arg Lys Met Leu Asn Tyr Gln Gly Leu Gln Arg Val Lys Ile Ile
    210                 215                 220

Ala Ser Asp Asn Leu Trp Glu Ser Ile Ser Ala Ser Met Leu Leu Asp
225                 230                 235                 240

Ala Glu Leu Phe Lys Val Val Asp Val Ile Gly Ala His Tyr Pro Gly
                245                 250                 255

Thr His Ser Ala Lys Asp Ala Lys Leu Thr Gly Lys Lys Leu Trp Ser
            260                 265                 270

Ser Glu Asp Phe Ser Thr Leu Asn Ser Asp Met Gly Ala Gly Cys Trp
        275                 280                 285

Gly Arg Ile Leu Asn Gln Asn Tyr Ile Asn Gly Tyr Met Thr Ser Thr
    290                 295                 300

Ile Ala Trp Asn Leu Val Ala Ser Tyr Tyr Glu Gln Leu Pro Tyr Gly
305                 310                 315                 320

Arg Cys Gly Leu Met Thr Ala Gln Glu Pro Trp Ser Gly His Tyr Val
                325                 330                 335

Val Glu Ser Pro Val Trp Val Ser Ala His Thr Thr Gln Phe Thr Gln
            340                 345                 350

Pro Gly Trp Tyr Tyr Leu Lys Thr Val Gly His Leu Glu Lys Gly Gly
        355                 360                 365

Ser Tyr Val Ala Leu Thr Asp Gly Leu Gly Asn Leu Thr Ile Ile Ile
    370                 375                 380

Glu Thr Met Ser His Lys His Ser Lys Cys Ile Arg Pro Phe Leu Pro
385                 390                 395                 400

Tyr Phe Asn Val Ser Gln Gln Phe Ala Thr Phe Val Leu Lys Gly Ser
                405                 410                 415

Phe Ser Glu Ile Pro Glu Leu Gln Val Trp Tyr Thr Lys Leu Gly Lys
            420                 425                 430

Thr Ser Glu Arg Phe Leu Phe Lys Gln Leu Asp Ser Leu Trp Leu Leu
        435                 440                 445

Asp Ser Asp Gly Ser Phe Thr Leu Ser Leu His Glu Asp Glu Leu Phe
    450                 455                 460

Thr Leu Thr Thr Leu Thr Thr Gly Arg Lys Gly Ser Tyr Pro Leu Pro
465                 470                 475                 480

Pro Lys Ser Gln Pro Phe Pro Ser Thr Tyr Lys Asp Asp Phe Asn Val
                485                 490                 495

Asp Tyr Pro Phe Phe Ser Glu Ala Pro Asn Phe Ala Asp Gln Thr Gly
            500                 505                 510

Val Phe Glu Tyr Phe Thr Asn Ile Glu Asp Pro Gly Glu His His Phe
```

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```
        515                 520                 525

Thr Leu Arg Gln Val Leu Asn Gln Arg Pro Ile Thr Trp Ala Ala Asp
    530                 535                 540

Ala Ser Asn Thr Ile Ser Ile Ile Gly Asp Tyr Asn Trp Thr Asn Leu
545                 550                 555                 560

Thr Ile Lys Cys Asp Val Tyr Ile Glu Thr Pro Asp Thr Gly Gly Val
                565                 570                 575

Phe Ile Ala Gly Arg Val Asn Lys Gly Gly Ile Leu Ile Arg Ser Ala
            580                 585                 590

Arg Gly Ile Phe Phe Trp Ile Phe Ala Asn Gly Ser Tyr Arg Val Thr
        595                 600                 605

Gly Asp Leu Ala Gly Trp Ile Ile Tyr Ala Leu Gly Arg Val Glu Val
    610                 615                 620

Thr Ala Lys Lys Trp Tyr Thr Leu Thr Leu Thr Ile Lys Gly His Phe
625                 630                 635                 640

Thr Ser Gly Met Leu Asn Asp Lys Ser Leu Trp Thr Asp Ile Pro Val
                645                 650                 655

Asn Phe Pro Lys Asn Gly Trp Ala Ala Ile Gly Thr His Ser Phe Glu
            660                 665                 670

Phe Ala Gln Phe Asp Asn Phe Leu Val Glu Ala Thr Arg
        675                 680                 685


<210> SEQ ID NO 12
<211> LENGTH: 1011
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Gly Ala Tyr Ala Arg Ala Ser Gly Val Cys Ala Arg Gly Cys Leu
1               5                   10                  15

Asp Ser Ala Gly Pro Trp Thr Met Ser Arg Ala Leu Arg Pro Pro Leu
            20                  25                  30

Pro Pro Leu Cys Phe Phe Leu Leu Leu Leu Ala Ala Ala Gly Ala Arg
        35                  40                  45

Ala Gly Gly Tyr Glu Thr Cys Pro Thr Val Gln Pro Asn Met Leu Asn
    50                  55                  60

Val His Leu Leu Pro His Thr His Asp Asp Val Gly Trp Leu Lys Thr
65                  70                  75                  80

Val Asp Gln Tyr Phe Tyr Gly Ile Lys Asn Asp Ile Gln His Ala Gly
                85                  90                  95

Val Gln Tyr Ile Leu Asp Ser Val Ile Ser Ala Leu Leu Ala Asp Pro
            100                 105                 110

Thr Arg Arg Phe Ile Tyr Val Glu Ile Ala Phe Phe Ser Arg Trp Trp
        115                 120                 125

His Gln Gln Thr Asn Ala Thr Gln Glu Val Val Arg Asp Leu Val Arg
    130                 135                 140

Gln Gly Arg Leu Glu Phe Ala Asn Gly Gly Trp Val Met Asn Asp Glu
145                 150                 155                 160

Ala Ala Thr His Tyr Gly Ala Ile Val Asp Gln Met Thr Leu Gly Leu
                165                 170                 175

Arg Phe Leu Glu Asp Thr Phe Gly Asn Asp Gly Arg Pro Arg Val Ala
            180                 185                 190
```

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```
Trp His Ile Asp Pro Phe Gly His Ser Arg Glu Gln Ala Ser Leu Phe
        195                 200                 205

Ala Gln Met Gly Phe Asp Gly Phe Phe Phe Gly Arg Leu Asp Tyr Gln
    210                 215                 220

Asp Lys Trp Val Arg Met Gln Lys Leu Glu Met Glu Gln Val Trp Arg
225                 230                 235                 240

Ala Ser Thr Ser Leu Lys Pro Pro Thr Ala Asp Leu Phe Thr Gly Val
                245                 250                 255

Leu Pro Asn Gly Tyr Asn Pro Pro Arg Asn Leu Cys Trp Asp Val Leu
            260                 265                 270

Cys Val Asp Gln Pro Leu Val Glu Asp Pro Arg Ser Pro Glu Tyr Asn
        275                 280                 285

Ala Lys Glu Leu Val Asp Tyr Phe Leu Asn Val Ala Thr Ala Gln Gly
    290                 295                 300

Arg Tyr Tyr Arg Thr Asn His Thr Val Met Thr Met Gly Ser Asp Phe
305                 310                 315                 320

Gln Tyr Glu Asn Ala Asn Met Trp Phe Lys Asn Leu Asp Lys Leu Ile
                325                 330                 335

Arg Leu Val Asn Ala Gln Gln Ala Lys Gly Ser Ser Val His Val Leu
            340                 345                 350

Tyr Ser Thr Pro Ala Cys Tyr Leu Trp Glu Leu Asn Lys Ala Asn Leu
        355                 360                 365

Thr Trp Ser Val Lys His Asp Asp Phe Phe Pro Tyr Ala Asp Gly Pro
    370                 375                 380

His Gln Phe Trp Thr Gly Tyr Phe Ser Ser Arg Pro Ala Leu Lys Arg
385                 390                 395                 400

Tyr Glu Arg Leu Ser Tyr Asn Phe Leu Gln Val Cys Asn Gln Leu Glu
                405                 410                 415

Ala Leu Val Gly Leu Ala Ala Asn Val Gly Pro Tyr Gly Ser Gly Asp
            420                 425                 430

Ser Ala Pro Leu Asn Glu Ala Met Ala Val Leu Gln His His Asp Ala
        435                 440                 445

Val Ser Gly Thr Ser Arg Gln His Val Ala Asn Asp Tyr Ala Arg Gln
    450                 455                 460

Leu Ala Ala Gly Trp Gly Pro Cys Glu Val Leu Leu Ser Asn Ala Leu
465                 470                 475                 480

Ala Arg Leu Arg Gly Phe Lys Asp His Phe Thr Phe Cys Gln Gln Leu
                485                 490                 495

Asn Ile Ser Ile Cys Pro Leu Ser Gln Thr Ala Ala Arg Phe Gln Val
            500                 505                 510

Ile Val Tyr Asn Pro Leu Gly Arg Lys Val Asn Trp Met Val Arg Leu
        515                 520                 525

Pro Val Ser Glu Gly Val Phe Val Val Lys Asp Pro Asn Gly Arg Thr
    530                 535                 540

Val Pro Ser Asp Val Val Ile Phe Pro Ser Ser Asp Ser Gln Ala His
545                 550                 555                 560

Pro Pro Glu Leu Leu Phe Ser Ala Ser Leu Pro Ala Leu Gly Phe Ser
                565                 570                 575

Thr Tyr Ser Val Ala Gln Val Pro Arg Trp Lys Pro Gln Ala Arg Ala
            580                 585                 590

Pro Gln Pro Ile Pro Arg Arg Ser Trp Ser Pro Ala Leu Thr Ile Glu
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```
        595                 600                 605

Asn Glu His Ile Arg Ala Thr Phe Asp Pro Asp Thr Gly Leu Leu Met
    610                 615                 620

Glu Ile Met Asn Met Asn Gln Gln Leu Leu Leu Pro Val Arg Gln Thr
625                 630                 635                 640

Phe Phe Trp Tyr Asn Ala Ser Ile Gly Asp Asn Glu Ser Asp Gln Ala
                645                 650                 655

Ser Gly Ala Tyr Ile Phe Arg Pro Asn Gln Gln Lys Pro Leu Pro Val
            660                 665                 670

Ser Arg Trp Ala Gln Ile His Leu Val Lys Thr Pro Leu Val Gln Glu
        675                 680                 685

Val His Gln Asn Phe Ser Ala Trp Cys Ser Gln Val Val Arg Leu Tyr
    690                 695                 700

Pro Gly Gln Arg His Leu Glu Leu Glu Trp Ser Val Gly Pro Ile Pro
705                 710                 715                 720

Val Gly Asp Thr Trp Gly Lys Glu Val Ile Ser Arg Phe Asp Thr Pro
                725                 730                 735

Leu Glu Thr Lys Gly Arg Phe Tyr Thr Asp Ser Asn Gly Arg Glu Ile
            740                 745                 750

Leu Glu Arg Arg Arg Asp Tyr Arg Pro Thr Trp Lys Leu Asn Gln Thr
        755                 760                 765

Glu Pro Val Ala Gly Asn Tyr Tyr Pro Val Asn Thr Arg Ile Tyr Ile
    770                 775                 780

Thr Asp Gly Asn Met Gln Leu Thr Val Leu Thr Asp Arg Ser Gln Gly
785                 790                 795                 800

Gly Ser Ser Leu Arg Asp Gly Ser Leu Glu Leu Met Val His Arg Arg
                805                 810                 815

Leu Leu Lys Asp Asp Gly Arg Gly Val Ser Glu Pro Leu Met Glu Asn
            820                 825                 830

Gly Ser Gly Ala Trp Val Arg Gly Arg His Leu Val Leu Leu Asp Thr
        835                 840                 845

Ala Gln Ala Ala Ala Ala Gly His Arg Leu Leu Ala Glu Gln Glu Val
    850                 855                 860

Leu Ala Pro Gln Val Val Leu Ala Pro Gly Gly Gly Ala Ala Tyr Asn
865                 870                 875                 880

Leu Gly Ala Pro Pro Arg Thr Gln Phe Ser Gly Leu Arg Arg Asp Leu
                885                 890                 895

Pro Pro Ser Val His Leu Leu Thr Leu Ala Ser Trp Gly Pro Glu Met
            900                 905                 910

Val Leu Leu Arg Leu Glu His Gln Phe Ala Val Gly Glu Asp Ser Gly
        915                 920                 925

Arg Asn Leu Ser Ala Pro Val Thr Leu Asn Leu Arg Asp Leu Phe Ser
    930                 935                 940

Thr Phe Thr Ile Thr Arg Leu Gln Glu Thr Thr Leu Val Ala Asn Gln
945                 950                 955                 960

Leu Arg Glu Ala Ala Ser Arg Leu Lys Trp Thr Thr Asn Thr Gly Pro
                965                 970                 975

Thr Pro His Gln Thr Pro Tyr Gln Leu Asp Pro Ala Asn Ile Thr Leu
            980                 985                 990

Glu Pro Met Glu Ile Arg Thr Phe  Leu Ala Ser Val Gln  Trp Lys Glu
        995                 1000                1005
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Val Asp Gly
1010

<210> SEQ ID NO 13
<211> LENGTH: 879
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Met Arg Leu His Leu Leu Leu Leu Leu Ala Leu Cys Gly Ala Gly Thr
1 5 10 15

Thr Ala Ala Glu Leu Ser Tyr Ser Leu Arg Gly Asn Trp Ser Ile Cys
20 25 30

Asn Gly Asn Gly Ser Leu Glu Leu Pro Gly Ala Val Pro Gly Cys Val
35 40 45

His Ser Ala Leu Phe Gln Gln Gly Leu Ile Gln Asp Ser Tyr Tyr Arg
50 55 60

Phe Asn Asp Leu Asn Tyr Arg Trp Val Ser Leu Asp Asn Trp Thr Tyr
65 70 75 80

Ser Lys Glu Phe Lys Ile Pro Phe Glu Ile Ser Lys Trp Gln Lys Val
85 90 95

Asn Leu Ile Leu Glu Gly Val Asp Thr Val Ser Lys Ile Leu Phe Asn
100 105 110

Glu Val Thr Ile Gly Glu Thr Asp Asn Met Phe Asn Arg Tyr Ser Phe
115 120 125

Asp Ile Thr Asn Val Val Arg Asp Val Asn Ser Ile Glu Leu Arg Phe
130 135 140

Gln Ser Ala Val Leu Tyr Ala Ala Gln Gln Ser Lys Ala His Thr Arg
145 150 155 160

Tyr Gln Val Pro Pro Asp Cys Pro Pro Leu Val Gln Lys Gly Glu Cys
165 170 175

His Val Asn Phe Val Arg Lys Glu Gln Cys Ser Phe Ser Trp Asp Trp
180 185 190

Gly Pro Ser Phe Pro Thr Gln Gly Ile Trp Lys Asp Val Arg Ile Glu
195 200 205

Ala Tyr Asn Ile Cys His Leu Asn Tyr Phe Thr Phe Ser Pro Ile Tyr
210 215 220

Asp Lys Ser Ala Gln Glu Trp Asn Leu Glu Ile Glu Ser Thr Phe Asp
225 230 235 240

Val Val Ser Ser Lys Pro Val Gly Gly Gln Val Ile Val Ala Ile Pro
245 250 255

Lys Leu Gln Thr Gln Gln Thr Tyr Ser Ile Glu Leu Gln Pro Gly Lys
260 265 270

Arg Ile Val Glu Leu Phe Val Asn Ile Ser Lys Asn Ile Thr Val Glu
275 280 285

Thr Trp Trp Pro His Gly His Gly Asn Gln Thr Gly Tyr Asn Met Thr
290 295 300

Val Leu Phe Glu Leu Asp Gly Gly Leu Asn Ile Glu Lys Ser Ala Lys
305 310 315 320

Val Tyr Phe Arg Thr Val Glu Leu Ile Glu Glu Pro Ile Lys Gly Ser
325 330 335

Pro Gly Leu Ser Phe Tyr Phe Lys Ile Asn Gly Phe Pro Ile Phe Leu

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            340                 345                 350

Lys Gly Ser Asn Trp Ile Pro Ala Asp Ser Phe Gln Asp Arg Val Thr
        355                 360                 365

Ser Glu Leu Leu Arg Leu Leu Leu Gln Ser Val Val Asp Ala Asn Met
    370                 375                 380

Asn Thr Leu Arg Val Trp Gly Gly Gly Ile Tyr Glu Gln Asp Glu Phe
385                 390                 395                 400

Tyr Glu Leu Cys Asp Glu Leu Gly Ile Met Val Trp Gln Asp Phe Met
                405                 410                 415

Phe Ala Cys Ala Leu Tyr Pro Thr Asp Gln Gly Phe Leu Asp Ser Val
            420                 425                 430

Thr Ala Glu Val Ala Tyr Gln Ile Lys Arg Leu Lys Ser His Pro Ser
        435                 440                 445

Ile Ile Ile Trp Ser Gly Asn Asn Glu Asn Glu Glu Ala Leu Met Met
    450                 455                 460

Asn Trp Tyr His Ile Ser Phe Thr Asp Arg Pro Ile Tyr Ile Lys Asp
465                 470                 475                 480

Tyr Val Thr Leu Tyr Val Lys Asn Ile Arg Glu Leu Val Leu Ala Gly
                485                 490                 495

Asp Lys Ser Arg Pro Phe Ile Thr Ser Ser Pro Thr Asn Gly Ala Glu
            500                 505                 510

Thr Val Ala Glu Ala Trp Val Ser Gln Asn Pro Asn Ser Asn Tyr Phe
        515                 520                 525

Gly Asp Val His Phe Tyr Asp Tyr Ile Ser Asp Cys Trp Asn Trp Lys
    530                 535                 540

Val Phe Pro Lys Ala Arg Phe Ala Ser Glu Tyr Gly Tyr Gln Ser Trp
545                 550                 555                 560

Pro Ser Phe Ser Thr Leu Glu Lys Val Ser Ser Thr Glu Asp Trp Ser
                565                 570                 575

Phe Asn Ser Lys Phe Ser Leu His Arg Gln His His Glu Gly Gly Asn
            580                 585                 590

Lys Gln Met Leu Tyr Gln Ala Gly Leu His Phe Lys Leu Pro Gln Ser
        595                 600                 605

Thr Asp Pro Leu Arg Thr Phe Lys Asp Thr Ile Tyr Leu Thr Gln Val
    610                 615                 620

Met Gln Ala Gln Cys Val Lys Thr Glu Thr Glu Phe Tyr Arg Arg Ser
625                 630                 635                 640

Arg Ser Glu Ile Val Asp Gln Gln Gly His Thr Met Gly Ala Leu Tyr
                645                 650                 655

Trp Gln Leu Asn Asp Ile Trp Gln Ala Pro Ser Trp Ala Ser Leu Glu
            660                 665                 670

Tyr Gly Gly Lys Trp Lys Met Leu His Tyr Phe Ala Gln Asn Phe Phe
        675                 680                 685

Ala Pro Leu Leu Pro Val Gly Phe Glu Asn Glu Asn Thr Phe Tyr Ile
    690                 695                 700

Tyr Gly Val Ser Asp Leu His Ser Asp Tyr Ser Met Thr Leu Ser Val
705                 710                 715                 720

Arg Val His Thr Trp Ser Ser Leu Glu Pro Val Cys Ser Arg Val Thr
                725                 730                 735

Glu Arg Phe Val Met Lys Gly Gly Glu Ala Val Cys Leu Tyr Glu Glu
            740                 745                 750
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Pro Val Ser Glu Leu Leu Arg Arg Cys Gly Asn Cys Thr Arg Glu Ser
        755                 760                 765

Cys Val Val Ser Phe Tyr Leu Ser Ala Asp His Glu Leu Leu Ser Pro
    770                 775                 780

Thr Asn Tyr His Phe Leu Ser Ser Pro Lys Glu Ala Val Gly Leu Cys
785                 790                 795                 800

Lys Ala Gln Ile Thr Ala Ile Ile Ser Gln Gln Gly Asp Ile Phe Val
                805                 810                 815

Phe Asp Leu Glu Thr Ser Ala Val Ala Pro Phe Val Trp Leu Asp Val
            820                 825                 830

Gly Ser Ile Pro Gly Arg Phe Ser Asp Asn Gly Phe Leu Met Thr Glu
        835                 840                 845

Lys Thr Arg Thr Ile Leu Phe Tyr Pro Trp Glu Pro Thr Ser Lys Asn
    850                 855                 860

Glu Leu Glu Gln Ser Phe His Val Thr Ser Leu Thr Asp Ile Tyr
865                 870                 875


<210> SEQ ID NO 14
<211> LENGTH: 507
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Met Gly Ala Pro Arg Ser Leu Leu Leu Ala Leu Ala Ala Gly Leu Ala
1               5                   10                  15

Val Ala Arg Pro Pro Asn Ile Val Leu Ile Phe Ala Asp Asp Leu Gly
            20                  25                  30

Tyr Gly Asp Leu Gly Cys Tyr Gly His Pro Ser Ser Thr Thr Pro Asn
        35                  40                  45

Leu Asp Gln Leu Ala Ala Gly Gly Leu Arg Phe Thr Asp Phe Tyr Val
    50                  55                  60

Pro Val Ser Leu Cys Thr Pro Ser Arg Ala Ala Leu Leu Thr Gly Arg
65                  70                  75                  80

Leu Pro Val Arg Met Gly Met Tyr Pro Gly Val Leu Val Pro Ser Ser
                85                  90                  95

Arg Gly Gly Leu Pro Leu Glu Glu Val Thr Val Ala Glu Val Leu Ala
            100                 105                 110

Ala Arg Gly Tyr Leu Thr Gly Met Ala Gly Lys Trp His Leu Gly Val
        115                 120                 125

Gly Pro Glu Gly Ala Phe Leu Pro Pro His Gln Gly Phe His Arg Phe
    130                 135                 140

Leu Gly Ile Pro Tyr Ser His Asp Gln Gly Pro Cys Gln Asn Leu Thr
145                 150                 155                 160

Cys Phe Pro Pro Ala Thr Pro Cys Asp Gly Gly Cys Asp Gln Gly Leu
                165                 170                 175

Val Pro Ile Pro Leu Leu Ala Asn Leu Ser Val Glu Ala Gln Pro Pro
            180                 185                 190

Trp Leu Pro Gly Leu Glu Ala Arg Tyr Met Ala Phe Ala His Asp Leu
        195                 200                 205

Met Ala Asp Ala Gln Arg Gln Asp Arg Pro Phe Phe Leu Tyr Tyr Ala
    210                 215                 220

Ser His His Thr His Tyr Pro Gln Phe Ser Gly Gln Ser Phe Ala Glu
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225                 230                 235                 240

Arg Ser Gly Arg Gly Pro Phe Gly Asp Ser Leu Met Glu Leu Asp Ala
                245                 250                 255

Ala Val Gly Thr Leu Met Thr Ala Ile Gly Asp Leu Gly Leu Leu Glu
            260                 265                 270

Glu Thr Leu Val Ile Phe Thr Ala Asp Asn Gly Pro Glu Thr Met Arg
        275                 280                 285

Met Ser Arg Gly Gly Cys Ser Gly Leu Leu Arg Cys Gly Lys Gly Thr
    290                 295                 300

Thr Tyr Glu Gly Gly Val Arg Glu Pro Ala Leu Ala Phe Trp Pro Gly
305                 310                 315                 320

His Ile Ala Pro Gly Val Thr His Glu Leu Ala Ser Ser Leu Asp Leu
                325                 330                 335

Leu Pro Thr Leu Ala Ala Leu Ala Gly Ala Pro Leu Pro Asn Val Thr
            340                 345                 350

Leu Asp Gly Phe Asp Leu Ser Pro Leu Leu Leu Gly Thr Gly Lys Ser
        355                 360                 365

Pro Arg Gln Ser Leu Phe Phe Tyr Pro Ser Tyr Pro Asp Glu Val Arg
    370                 375                 380

Gly Val Phe Ala Val Arg Thr Gly Lys Tyr Lys Ala His Phe Phe Thr
385                 390                 395                 400

Gln Gly Ser Ala His Ser Asp Thr Thr Ala Asp Pro Ala Cys His Ala
                405                 410                 415

Ser Ser Ser Leu Thr Ala His Glu Pro Pro Leu Leu Tyr Asp Leu Ser
            420                 425                 430

Lys Asp Pro Gly Glu Asn Tyr Asn Leu Leu Gly Gly Val Ala Gly Ala
        435                 440                 445

Thr Pro Glu Val Leu Gln Ala Leu Lys Gln Leu Gln Leu Leu Lys Ala
    450                 455                 460

Gln Leu Asp Ala Ala Val Thr Phe Gly Pro Ser Gln Val Ala Arg Gly
465                 470                 475                 480

Glu Asp Pro Ala Leu Gln Ile Cys Cys His Pro Gly Cys Thr Pro Arg
                485                 490                 495

Pro Ala Cys Cys His Cys Pro Asp Pro His Ala
            500                 505


<210> SEQ ID NO 15
<211> LENGTH: 507
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Met Gly Ala Pro Arg Ser Leu Leu Leu Ala Leu Ala Ala Gly Leu Ala
1               5                   10                  15

Val Ala Arg Pro Pro Asn Ile Val Leu Ile Phe Ala Asp Asp Leu Gly
            20                  25                  30

Tyr Gly Asp Leu Gly Cys Tyr Gly His Pro Ser Ser Thr Thr Pro Asn
        35                  40                  45

Leu Asp Gln Leu Ala Ala Gly Gly Leu Arg Phe Thr Asp Phe Tyr Val
    50                  55                  60

Pro Val Ser Leu Cys Thr Pro Ser Arg Ala Ala Leu Leu Thr Gly Arg
65                  70                  75                  80
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Leu Pro Val Arg Met Gly Met Tyr Pro Gly Val Leu Val Pro Ser Ser
                85                  90                  95

Arg Gly Gly Leu Pro Leu Glu Glu Val Thr Val Ala Glu Val Leu Ala
            100                 105                 110

Ala Arg Gly Tyr Leu Thr Gly Met Ala Gly Lys Trp His Leu Gly Val
        115                 120                 125

Gly Pro Glu Gly Ala Phe Leu Pro Pro His Gln Gly Phe His Arg Phe
    130                 135                 140

Leu Gly Ile Pro Tyr Ser His Asp Gln Gly Pro Cys Gln Asn Leu Thr
145                 150                 155                 160

Cys Phe Pro Pro Ala Thr Pro Cys Asp Gly Gly Cys Asp Gln Gly Leu
                165                 170                 175

Val Pro Ile Pro Leu Leu Ala Asn Leu Ser Val Glu Ala Gln Pro Pro
            180                 185                 190

Trp Leu Pro Gly Leu Glu Ala Arg Tyr Met Ala Phe Ala His Asp Leu
        195                 200                 205

Met Ala Asp Ala Gln Arg Gln Asp Arg Pro Phe Phe Leu Tyr Tyr Ala
    210                 215                 220

Ser His His Thr His Tyr Pro Gln Phe Ser Gly Gln Ser Phe Ala Glu
225                 230                 235                 240

Arg Ser Gly Arg Gly Pro Phe Gly Asp Ser Leu Met Glu Leu Asp Ala
                245                 250                 255

Ala Val Gly Thr Leu Met Thr Ala Ile Gly Asp Leu Gly Leu Leu Glu
            260                 265                 270

Glu Thr Leu Val Ile Phe Thr Ala Asp Asn Gly Pro Glu Thr Met Arg
        275                 280                 285

Met Ser Arg Gly Gly Cys Ser Gly Leu Leu Arg Cys Gly Lys Gly Thr
    290                 295                 300

Thr Tyr Glu Gly Gly Val Arg Glu Pro Ala Leu Ala Phe Trp Pro Gly
305                 310                 315                 320

His Ile Ala Pro Gly Val Thr His Glu Leu Ala Ser Ser Leu Asp Leu
                325                 330                 335

Leu Pro Thr Leu Ala Ala Leu Ala Gly Ala Pro Leu Pro Asn Val Thr
            340                 345                 350

Leu Asp Gly Phe Asp Leu Ser Pro Leu Leu Leu Gly Thr Gly Lys Ser
        355                 360                 365

Pro Arg Gln Ser Leu Phe Phe Tyr Pro Ser Tyr Pro Asp Glu Val Arg
    370                 375                 380

Gly Val Phe Ala Val Arg Thr Gly Lys Tyr Lys Ala His Phe Phe Thr
385                 390                 395                 400

Gln Gly Ser Ala His Ser Asp Thr Thr Ala Asp Pro Ala Cys His Ala
                405                 410                 415

Ser Ser Ser Leu Thr Ala His Glu Pro Pro Leu Leu Tyr Asp Leu Ser
            420                 425                 430

Lys Asp Pro Gly Glu Asn Tyr Asn Leu Leu Gly Gly Val Ala Gly Ala
        435                 440                 445

Thr Pro Glu Val Leu Gln Ala Leu Lys Gln Leu Gln Leu Leu Lys Ala
    450                 455                 460

Gln Leu Asp Ala Ala Val Thr Phe Gly Pro Ser Gln Val Ala Arg Gly
465                 470                 475                 480

Glu Asp Pro Ala Leu Gln Ile Cys Cys His Pro Gly Cys Thr Pro Arg
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                485                 490                 495

Pro Ala Cys Cys His Cys Pro Asp Pro His Ala
            500                 505


<210> SEQ ID NO 16
<211> LENGTH: 522
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Met Ala Ala Val Val Ala Ala Thr Arg Trp Trp Gln Leu Leu Leu Val
1               5                   10                  15

Leu Ser Ala Ala Gly Met Gly Ala Ser Gly Ala Pro Gln Pro Pro Asn
            20                  25                  30

Ile Leu Leu Leu Leu Met Asp Asp Met Gly Trp Gly Asp Leu Gly Val
        35                  40                  45

Tyr Gly Glu Pro Ser Arg Glu Thr Pro Asn Leu Asp Arg Met Ala Ala
    50                  55                  60

Glu Gly Leu Leu Phe Pro Asn Phe Tyr Ser Ala Asn Pro Leu Cys Ser
65                  70                  75                  80

Pro Ser Arg Ala Ala Leu Leu Thr Gly Arg Leu Pro Ile Arg Asn Gly
                85                  90                  95

Phe Tyr Thr Thr Asn Ala His Ala Arg Asn Ala Tyr Thr Pro Gln Glu
            100                 105                 110

Ile Val Gly Gly Ile Pro Asp Ser Glu Gln Leu Leu Pro Glu Leu Leu
        115                 120                 125

Lys Lys Ala Gly Tyr Val Ser Lys Ile Val Gly Lys Trp His Leu Gly
    130                 135                 140

His Arg Pro Gln Phe His Pro Leu Lys His Gly Phe Asp Glu Trp Phe
145                 150                 155                 160

Gly Ser Pro Asn Cys His Phe Gly Pro Tyr Asp Asn Lys Ala Arg Pro
                165                 170                 175

Asn Ile Pro Val Tyr Arg Asp Trp Glu Met Val Gly Arg Tyr Tyr Glu
            180                 185                 190

Glu Phe Pro Ile Asn Leu Lys Thr Gly Glu Ala Asn Leu Thr Gln Ile
        195                 200                 205

Tyr Leu Gln Glu Ala Leu Asp Phe Ile Lys Arg Gln Ala Arg His His
    210                 215                 220

Pro Phe Phe Leu Tyr Trp Ala Val Asp Ala Thr His Ala Pro Val Tyr
225                 230                 235                 240

Ala Ser Lys Pro Phe Leu Gly Thr Ser Gln Arg Gly Arg Tyr Gly Asp
                245                 250                 255

Ala Val Arg Glu Ile Asp Asp Ser Ile Gly Lys Ile Leu Glu Leu Leu
            260                 265                 270

Gln Asp Leu His Val Ala Asp Asn Thr Phe Val Phe Phe Thr Ser Asp
        275                 280                 285

Asn Gly Ala Ala Leu Ile Ser Ala Pro Glu Gln Gly Gly Ser Asn Gly
    290                 295                 300

Pro Phe Leu Cys Gly Lys Gln Thr Thr Phe Glu Gly Gly Met Arg Glu
305                 310                 315                 320

Pro Ala Leu Ala Trp Trp Pro Gly His Val Thr Ala Gly Gln Val Ser
                325                 330                 335
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His Gln Leu Gly Ser Ile Met Asp Leu Phe Thr Thr Ser Leu Ala Leu
            340                 345                 350

Ala Gly Leu Thr Pro Pro Ser Asp Arg Ala Ile Asp Gly Leu Asn Leu
        355                 360                 365

Leu Pro Thr Leu Leu Gln Gly Arg Leu Met Asp Arg Pro Ile Phe Tyr
    370                 375                 380

Tyr Arg Gly Asp Thr Leu Met Ala Ala Thr Leu Gly Gln His Lys Ala
385                 390                 395                 400

His Phe Trp Thr Trp Thr Asn Ser Trp Glu Asn Phe Arg Gln Gly Ile
                405                 410                 415

Asp Phe Cys Pro Gly Gln Asn Val Ser Gly Val Thr Thr His Asn Leu
            420                 425                 430

Glu Asp His Thr Lys Leu Pro Leu Ile Phe His Leu Gly Arg Asp Pro
        435                 440                 445

Gly Glu Arg Phe Pro Leu Ser Phe Ala Ser Ala Glu Tyr Gln Glu Ala
    450                 455                 460

Leu Ser Arg Ile Thr Ser Val Val Gln Gln His Gln Glu Ala Leu Val
465                 470                 475                 480

Pro Ala Gln Pro Gln Leu Asn Val Cys Asn Trp Ala Val Met Asn Trp
                485                 490                 495

Ala Pro Pro Gly Cys Glu Lys Leu Gly Lys Cys Leu Thr Pro Pro Glu
            500                 505                 510

Ser Ile Pro Lys Lys Cys Leu Trp Ser His
        515                 520


<210> SEQ ID NO 17
<211> LENGTH: 631
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Met Pro Arg Tyr Gly Ala Ser Leu Arg Gln Ser Cys Pro Arg Ser Gly
1               5                   10                  15

Arg Glu Gln Gly Gln Asp Gly Thr Ala Gly Ala Pro Gly Leu Leu Trp
            20                  25                  30

Met Gly Leu Val Leu Ala Leu Ala Leu Ala Leu Ala Leu Ala Leu Ala
        35                  40                  45

Leu Ser Asp Ser Arg Val Leu Trp Ala Pro Ala Glu Ala His Pro Leu
    50                  55                  60

Ser Pro Gln Gly His Pro Ala Arg Leu His Arg Ile Val Pro Arg Leu
65                  70                  75                  80

Arg Asp Val Phe Gly Trp Gly Asn Leu Thr Cys Pro Ile Cys Lys Gly
                85                  90                  95

Leu Phe Thr Ala Ile Asn Leu Gly Leu Lys Lys Glu Pro Asn Val Ala
            100                 105                 110

Arg Val Gly Ser Val Ala Ile Lys Leu Cys Asn Leu Leu Lys Ile Ala
        115                 120                 125

Pro Pro Ala Val Cys Gln Ser Ile Val His Leu Phe Glu Asp Asp Met
    130                 135                 140

Val Glu Val Trp Arg Arg Ser Val Leu Ser Pro Ser Glu Ala Cys Gly
145                 150                 155                 160

Leu Leu Leu Gly Ser Thr Cys Gly His Trp Asp Ile Phe Ser Ser Trp
                165                 170                 175
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Asn Ile Ser Leu Pro Thr Val Pro Lys Pro Pro Pro Lys Pro Pro Ser
            180                 185                 190

Pro Pro Ala Pro Gly Ala Pro Val Ser Arg Ile Leu Phe Leu Thr Asp
        195                 200                 205

Leu His Trp Asp His Asp Tyr Leu Glu Gly Thr Asp Pro Asp Cys Ala
    210                 215                 220

Asp Pro Leu Cys Cys Arg Arg Gly Ser Gly Leu Pro Pro Ala Ser Arg
225                 230                 235                 240

Pro Gly Ala Gly Tyr Trp Gly Glu Tyr Ser Lys Cys Asp Leu Pro Leu
                245                 250                 255

Arg Thr Leu Glu Ser Leu Leu Ser Gly Leu Gly Pro Ala Gly Pro Phe
            260                 265                 270

Asp Met Val Tyr Trp Thr Gly Asp Ile Pro Ala His Asp Val Trp His
        275                 280                 285

Gln Thr Arg Gln Asp Gln Leu Arg Ala Leu Thr Thr Val Thr Ala Leu
    290                 295                 300

Val Arg Lys Phe Leu Gly Pro Val Pro Val Tyr Pro Ala Val Gly Asn
305                 310                 315                 320

His Glu Ser Thr Pro Val Asn Ser Phe Pro Pro Pro Phe Ile Glu Gly
                325                 330                 335

Asn His Ser Ser Arg Trp Leu Tyr Glu Ala Met Ala Lys Ala Trp Glu
            340                 345                 350

Pro Trp Leu Pro Ala Glu Ala Leu Arg Thr Leu Arg Ile Gly Gly Phe
        355                 360                 365

Tyr Ala Leu Ser Pro Tyr Pro Gly Leu Arg Leu Ile Ser Leu Asn Met
    370                 375                 380

Asn Phe Cys Ser Arg Glu Asn Phe Trp Leu Leu Ile Asn Ser Thr Asp
385                 390                 395                 400

Pro Ala Gly Gln Leu Gln Trp Leu Val Gly Glu Leu Gln Ala Ala Glu
                405                 410                 415

Asp Arg Gly Asp Lys Val His Ile Ile Gly His Ile Pro Pro Gly His
            420                 425                 430

Cys Leu Lys Ser Trp Ser Trp Asn Tyr Tyr Arg Ile Val Ala Arg Tyr
        435                 440                 445

Glu Asn Thr Leu Ala Ala Gln Phe Phe Gly His Thr His Val Asp Glu
    450                 455                 460

Phe Glu Val Phe Tyr Asp Glu Glu Thr Leu Ser Arg Pro Leu Ala Val
465                 470                 475                 480

Ala Phe Leu Ala Pro Ser Ala Thr Thr Tyr Ile Gly Leu Asn Pro Gly
                485                 490                 495

Tyr Arg Val Tyr Gln Ile Asp Gly Asn Tyr Ser Gly Ser Ser His Val
            500                 505                 510

Val Leu Asp His Glu Thr Tyr Ile Leu Asn Leu Thr Gln Ala Asn Ile
        515                 520                 525

Pro Gly Ala Ile Pro His Trp Gln Leu Leu Tyr Arg Ala Arg Glu Thr
    530                 535                 540

Tyr Gly Leu Pro Asn Thr Leu Pro Thr Ala Trp His Asn Leu Val Tyr
545                 550                 555                 560

Arg Met Arg Gly Asp Met Gln Leu Phe Gln Thr Phe Trp Phe Leu Tyr
                565                 570                 575
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His Lys Gly His Pro Pro Ser Glu Pro Cys Gly Thr Pro Cys Arg Leu
            580                 585                 590

Ala Thr Leu Cys Ala Gln Leu Ser Ala Arg Ala Asp Ser Pro Ala Leu
        595                 600                 605

Cys Arg His Leu Met Pro Asp Gly Ser Leu Pro Glu Ala Gln Ser Leu
    610                 615                 620

Trp Pro Arg Pro Leu Phe Cys
625                 630


<210> SEQ ID NO 18
<211> LENGTH: 952
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met Gly Val Arg His Pro Pro Cys Ser His Arg Leu Leu Ala Val Cys
1               5                   10                  15

Ala Leu Val Ser Leu Ala Thr Ala Ala Leu Leu Gly His Ile Leu Leu
            20                  25                  30

His Asp Phe Leu Leu Val Pro Arg Glu Leu Ser Gly Ser Ser Pro Val
        35                  40                  45

Leu Glu Glu Thr His Pro Ala His Gln Gln Gly Ala Ser Arg Pro Gly
    50                  55                  60

Pro Arg Asp Ala Gln Ala His Pro Gly Arg Pro Arg Ala Val Pro Thr
65                  70                  75                  80

Gln Cys Asp Val Pro Pro Asn Ser Arg Phe Asp Cys Ala Pro Asp Lys
                85                  90                  95

Ala Ile Thr Gln Glu Gln Cys Glu Ala Arg Gly Cys Cys Tyr Ile Pro
            100                 105                 110

Ala Lys Gln Gly Leu Gln Gly Ala Gln Met Gly Gln Pro Trp Cys Phe
        115                 120                 125

Phe Pro Pro Ser Tyr Pro Ser Tyr Lys Leu Glu Asn Leu Ser Ser Ser
    130                 135                 140

Glu Met Gly Tyr Thr Ala Thr Leu Thr Arg Thr Thr Pro Thr Phe Phe
145                 150                 155                 160

Pro Lys Asp Ile Leu Thr Leu Arg Leu Asp Val Met Met Glu Thr Glu
                165                 170                 175

Asn Arg Leu His Phe Thr Ile Lys Asp Pro Ala Asn Arg Arg Tyr Glu
            180                 185                 190

Val Pro Leu Glu Thr Pro His Val His Ser Arg Ala Pro Ser Pro Leu
        195                 200                 205

Tyr Ser Val Glu Phe Ser Glu Glu Pro Phe Gly Val Ile Val Arg Arg
    210                 215                 220

Gln Leu Asp Gly Arg Val Leu Leu Asn Thr Thr Val Ala Pro Leu Phe
225                 230                 235                 240

Phe Ala Asp Gln Phe Leu Gln Leu Ser Thr Ser Leu Pro Ser Gln Tyr
                245                 250                 255

Ile Thr Gly Leu Ala Glu His Leu Ser Pro Leu Met Leu Ser Thr Ser
            260                 265                 270

Trp Thr Arg Ile Thr Leu Trp Asn Arg Asp Leu Ala Pro Thr Pro Gly
        275                 280                 285

Ala Asn Leu Tyr Gly Ser His Pro Phe Tyr Leu Ala Leu Glu Asp Gly
    290                 295                 300
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Gly Ser Ala His Gly Val Phe Leu Leu Asn Ser Asn Ala Met Asp Val
305                 310                 315                 320

Val Leu Gln Pro Ser Pro Ala Leu Ser Trp Arg Ser Thr Gly Gly Ile
                325                 330                 335

Leu Asp Val Tyr Ile Phe Leu Gly Pro Glu Pro Lys Ser Val Val Gln
            340                 345                 350

Gln Tyr Leu Asp Val Val Gly Tyr Pro Phe Met Pro Pro Tyr Trp Gly
        355                 360                 365

Leu Gly Phe His Leu Cys Arg Trp Gly Tyr Ser Ser Thr Ala Ile Thr
    370                 375                 380

Arg Gln Val Val Glu Asn Met Thr Arg Ala His Phe Pro Leu Asp Val
385                 390                 395                 400

Gln Trp Asn Asp Leu Asp Tyr Met Asp Ser Arg Arg Asp Phe Thr Phe
                405                 410                 415

Asn Lys Asp Gly Phe Arg Asp Phe Pro Ala Met Val Gln Glu Leu His
            420                 425                 430

Gln Gly Gly Arg Arg Tyr Met Met Ile Val Asp Pro Ala Ile Ser Ser
        435                 440                 445

Ser Gly Pro Ala Gly Ser Tyr Arg Pro Tyr Asp Glu Gly Leu Arg Arg
    450                 455                 460

Gly Val Phe Ile Thr Asn Glu Thr Gly Gln Pro Leu Ile Gly Lys Val
465                 470                 475                 480

Trp Pro Gly Ser Thr Ala Phe Pro Asp Phe Thr Asn Pro Thr Ala Leu
                485                 490                 495

Ala Trp Trp Glu Asp Met Val Ala Glu Phe His Asp Gln Val Pro Phe
            500                 505                 510

Asp Gly Met Trp Ile Asp Met Asn Glu Pro Ser Asn Phe Ile Arg Gly
        515                 520                 525

Ser Glu Asp Gly Cys Pro Asn Asn Glu Leu Glu Asn Pro Pro Tyr Val
    530                 535                 540

Pro Gly Val Val Gly Gly Thr Leu Gln Ala Ala Thr Ile Cys Ala Ser
545                 550                 555                 560

Ser His Gln Phe Leu Ser Thr His Tyr Asn Leu His Asn Leu Tyr Gly
                565                 570                 575

Leu Thr Glu Ala Ile Ala Ser His Arg Ala Leu Val Lys Ala Arg Gly
            580                 585                 590

Thr Arg Pro Phe Val Ile Ser Arg Ser Thr Phe Ala Gly His Gly Arg
        595                 600                 605

Tyr Ala Gly His Trp Thr Gly Asp Val Trp Ser Ser Trp Glu Gln Leu
    610                 615                 620

Ala Ser Ser Val Pro Glu Ile Leu Gln Phe Asn Leu Leu Gly Val Pro
625                 630                 635                 640

Leu Val Gly Ala Asp Val Cys Gly Phe Leu Gly Asn Thr Ser Glu Glu
                645                 650                 655

Leu Cys Val Arg Trp Thr Gln Leu Gly Ala Phe Tyr Pro Phe Met Arg
            660                 665                 670

Asn His Asn Ser Leu Leu Ser Leu Pro Gln Glu Pro Tyr Ser Phe Ser
        675                 680                 685

Glu Pro Ala Gln Gln Ala Met Arg Lys Ala Leu Thr Leu Arg Tyr Ala
    690                 695                 700
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Leu Leu Pro His Leu Tyr Thr Leu Phe His Gln Ala His Val Ala Gly
705                 710                 715                 720

Glu Thr Val Ala Arg Pro Leu Phe Leu Glu Phe Pro Lys Asp Ser Ser
                725                 730                 735

Thr Trp Thr Val Asp His Gln Leu Leu Trp Gly Glu Ala Leu Leu Ile
            740                 745                 750

Thr Pro Val Leu Gln Ala Gly Lys Ala Glu Val Thr Gly Tyr Phe Pro
        755                 760                 765

Leu Gly Thr Trp Tyr Asp Leu Gln Thr Val Pro Val Glu Ala Leu Gly
    770                 775                 780

Ser Leu Pro Pro Pro Pro Ala Ala Pro Arg Glu Pro Ala Ile His Ser
785                 790                 795                 800

Glu Gly Gln Trp Val Thr Leu Pro Ala Pro Leu Asp Thr Ile Asn Val
                805                 810                 815

His Leu Arg Ala Gly Tyr Ile Ile Pro Leu Gln Gly Pro Gly Leu Thr
            820                 825                 830

Thr Thr Glu Ser Arg Gln Gln Pro Met Ala Leu Ala Val Ala Leu Thr
        835                 840                 845

Lys Gly Gly Glu Ala Arg Gly Glu Leu Phe Trp Asp Asp Gly Glu Ser
    850                 855                 860

Leu Glu Val Leu Glu Arg Gly Ala Tyr Thr Gln Val Ile Phe Leu Ala
865                 870                 875                 880

Arg Asn Asn Thr Ile Val Asn Glu Leu Val Arg Val Thr Ser Glu Gly
                885                 890                 895

Ala Gly Leu Gln Leu Gln Lys Val Thr Val Leu Gly Val Ala Thr Ala
            900                 905                 910

Pro Gln Gln Val Leu Ser Asn Gly Val Pro Val Ser Asn Phe Thr Tyr
        915                 920                 925

Ser Pro Asp Thr Lys Val Leu Asp Ile Cys Val Ser Leu Leu Met Gly
    930                 935                 940

Glu Gln Phe Leu Val Ser Trp Cys
945                 950


<210> SEQ ID NO 19
<211> LENGTH: 556
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Met Glu Leu Cys Gly Leu Gly Leu Pro Arg Pro Pro Met Leu Leu Ala
1               5                   10                  15

Leu Leu Leu Ala Thr Leu Leu Ala Ala Met Leu Ala Leu Leu Thr Gln
            20                  25                  30

Val Ala Leu Val Val Gln Val Ala Glu Ala Ala Arg Ala Pro Ser Val
        35                  40                  45

Ser Ala Lys Pro Gly Pro Ala Leu Trp Pro Leu Pro Leu Leu Val Lys
    50                  55                  60

Met Thr Pro Asn Leu Leu His Leu Ala Pro Glu Asn Phe Tyr Ile Ser
65                  70                  75                  80

His Ser Pro Asn Ser Thr Ala Gly Pro Ser Cys Thr Leu Leu Glu Glu
                85                  90                  95

Ala Phe Arg Arg Tyr His Gly Tyr Ile Phe Gly Phe Tyr Lys Trp His
            100                 105                 110
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His Glu Pro Ala Glu Phe Gln Ala Lys Thr Gln Val Gln Gln Leu Leu
        115                 120                 125

Val Ser Ile Thr Leu Gln Ser Glu Cys Asp Ala Phe Pro Asn Ile Ser
    130                 135                 140

Ser Asp Glu Ser Tyr Thr Leu Leu Val Lys Glu Pro Val Ala Val Leu
145                 150                 155                 160

Lys Ala Asn Arg Val Trp Gly Ala Leu Arg Gly Leu Glu Thr Phe Ser
                165                 170                 175

Gln Leu Val Tyr Gln Asp Ser Tyr Gly Thr Phe Thr Ile Asn Glu Ser
            180                 185                 190

Thr Ile Ile Asp Ser Pro Arg Phe Ser His Arg Gly Ile Leu Ile Asp
        195                 200                 205

Thr Ser Arg His Tyr Leu Pro Val Lys Ile Ile Leu Lys Thr Leu Asp
    210                 215                 220

Ala Met Ala Phe Asn Lys Phe Asn Val Leu His Trp His Ile Val Asp
225                 230                 235                 240

Asp Gln Ser Phe Pro Tyr Gln Ser Ile Thr Phe Pro Glu Leu Ser Asn
                245                 250                 255

Lys Gly Ser Tyr Ser Leu Ser His Val Tyr Thr Pro Asn Asp Val Arg
            260                 265                 270

Met Val Ile Glu Tyr Ala Arg Leu Arg Gly Ile Arg Val Leu Pro Glu
        275                 280                 285

Phe Asp Thr Pro Gly His Thr Leu Ser Trp Gly Lys Gly Gln Lys Asp
    290                 295                 300

Leu Leu Thr Pro Cys Tyr Ser Arg Gln Asn Lys Leu Asp Ser Phe Gly
305                 310                 315                 320

Pro Ile Asn Pro Thr Leu Asn Thr Thr Tyr Ser Phe Leu Thr Thr Phe
                325                 330                 335

Phe Lys Glu Ile Ser Glu Val Phe Pro Asp Gln Phe Ile His Leu Gly
            340                 345                 350

Gly Asp Glu Val Glu Phe Lys Cys Trp Glu Ser Asn Pro Lys Ile Gln
        355                 360                 365

Asp Phe Met Arg Gln Lys Gly Phe Gly Thr Asp Phe Lys Lys Leu Glu
    370                 375                 380

Ser Phe Tyr Ile Gln Lys Val Leu Asp Ile Ile Ala Thr Ile Asn Lys
385                 390                 395                 400

Gly Ser Ile Val Trp Gln Glu Val Phe Asp Asp Lys Ala Lys Leu Ala
                405                 410                 415

Pro Gly Thr Ile Val Glu Val Trp Lys Asp Ser Ala Tyr Pro Glu Glu
            420                 425                 430

Leu Ser Arg Val Thr Ala Ser Gly Phe Pro Val Ile Leu Ser Ala Pro
        435                 440                 445

Trp Tyr Leu Asp Leu Ile Ser Tyr Gly Gln Asp Trp Arg Lys Tyr Tyr
    450                 455                 460

Lys Val Glu Pro Leu Asp Phe Gly Gly Thr Gln Lys Gln Lys Gln Leu
465                 470                 475                 480

Phe Ile Gly Gly Glu Ala Cys Leu Trp Gly Glu Tyr Val Asp Ala Thr
                485                 490                 495

Asn Leu Thr Pro Arg Leu Trp Pro Arg Ala Ser Ala Val Gly Glu Arg
            500                 505                 510
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Leu Trp Ser Ser Lys Asp Val Arg Asp Met Asp Asp Ala Tyr Asp Arg
        515                 520                 525

Leu Thr Arg His Arg Cys Arg Met Val Glu Arg Gly Ile Ala Ala Gln
    530                 535                 540

Pro Leu Tyr Ala Gly Tyr Cys Asn His Glu Asn Met
545                 550                 555


<210> SEQ ID NO 20
<211> LENGTH: 663
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met Thr Gly Ala Arg Ala Ser Ala Ala Glu Gln Arg Arg Ala Gly Arg
1               5                   10                  15

Ser Gly Gln Ala Arg Ala Ala Glu Arg Ala Ala Gly Met Ser Gly Ala
            20                  25                  30

Gly Arg Ala Leu Ala Ala Leu Leu Leu Ala Ala Ser Val Leu Ser Ala
        35                  40                  45

Ala Leu Leu Ala Pro Gly Gly Ser Ser Gly Arg Asp Ala Gln Ala Ala
    50                  55                  60

Pro Pro Arg Asp Leu Asp Lys Lys Arg His Ala Glu Leu Lys Met Asp
65                  70                  75                  80

Gln Ala Leu Leu Leu Ile His Asn Glu Leu Leu Trp Thr Asn Leu Thr
                85                  90                  95

Val Tyr Trp Lys Ser Glu Cys Cys Tyr His Cys Leu Phe Gln Val Leu
            100                 105                 110

Val Asn Val Pro Gln Ser Pro Lys Ala Gly Lys Pro Ser Ala Ala Ala
        115                 120                 125

Ala Ser Val Ser Thr Gln His Gly Ser Ile Leu Gln Leu Asn Asp Thr
    130                 135                 140

Leu Glu Glu Lys Glu Val Cys Arg Leu Glu Tyr Arg Phe Gly Glu Phe
145                 150                 155                 160

Gly Asn Tyr Ser Leu Leu Val Lys Asn Ile His Asn Gly Val Ser Glu
                165                 170                 175

Ile Ala Cys Asp Leu Ala Val Asn Glu Asp Pro Val Asp Ser Asn Leu
            180                 185                 190

Pro Val Ser Ile Ala Phe Leu Ile Gly Leu Ala Val Ile Ile Val Ile
        195                 200                 205

Ser Phe Leu Arg Leu Leu Leu Ser Leu Asp Asp Phe Asn Asn Trp Ile
    210                 215                 220

Ser Lys Ala Ile Ser Ser Arg Glu Thr Asp Arg Leu Ile Asn Ser Glu
225                 230                 235                 240

Leu Gly Ser Pro Ser Arg Thr Asp Pro Leu Asp Gly Asp Val Gln Pro
                245                 250                 255

Ala Thr Trp Arg Leu Ser Ala Leu Pro Pro Arg Leu Arg Ser Val Asp
            260                 265                 270

Thr Phe Arg Gly Ile Ala Leu Ile Leu Met Val Phe Val Asn Tyr Gly
        275                 280                 285

Gly Gly Lys Tyr Trp Tyr Phe Lys His Ala Ser Trp Asn Gly Leu Thr
    290                 295                 300

Val Ala Asp Leu Val Phe Pro Trp Phe Val Phe Ile Met Gly Ser Ser
305                 310                 315                 320
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Ile Phe Leu Ser Met Thr Ser Ile Leu Gln Arg Gly Cys Ser Lys Phe
                325                 330                 335

Arg Leu Leu Gly Lys Ile Ala Trp Arg Ser Phe Leu Leu Ile Cys Ile
            340                 345                 350

Gly Ile Ile Ile Val Asn Pro Asn Tyr Cys Leu Gly Pro Leu Ser Trp
        355                 360                 365

Asp Lys Val Arg Ile Pro Gly Val Leu Gln Arg Leu Gly Val Thr Tyr
    370                 375                 380

Phe Val Val Ala Val Leu Glu Leu Leu Phe Ala Lys Pro Val Pro Glu
385                 390                 395                 400

His Cys Ala Ser Glu Arg Ser Cys Leu Ser Leu Arg Asp Ile Thr Ser
                405                 410                 415

Ser Trp Pro Gln Trp Leu Leu Ile Leu Val Leu Glu Gly Leu Trp Leu
            420                 425                 430

Gly Leu Thr Phe Leu Leu Pro Val Pro Gly Cys Pro Thr Gly Tyr Leu
        435                 440                 445

Gly Pro Gly Gly Ile Gly Asp Phe Gly Lys Tyr Pro Asn Cys Thr Gly
    450                 455                 460

Gly Ala Ala Gly Tyr Ile Asp Arg Leu Leu Leu Gly Asp Asp His Leu
465                 470                 475                 480

Tyr Gln His Pro Ser Ser Ala Val Leu Tyr His Thr Glu Val Ala Tyr
                485                 490                 495

Asp Pro Glu Gly Ile Leu Gly Thr Ile Asn Ser Ile Val Met Ala Phe
            500                 505                 510

Leu Gly Val Gln Ala Gly Lys Ile Leu Leu Tyr Tyr Lys Ala Arg Thr
        515                 520                 525

Lys Asp Ile Leu Ile Arg Phe Thr Ala Trp Cys Cys Ile Leu Gly Leu
    530                 535                 540

Ile Ser Val Ala Leu Thr Lys Val Ser Glu Asn Glu Gly Phe Ile Pro
545                 550                 555                 560

Val Asn Lys Asn Leu Trp Ser Leu Ser Tyr Val Thr Thr Leu Ser Ser
                565                 570                 575

Phe Ala Phe Phe Ile Leu Leu Val Leu Tyr Pro Val Val Asp Val Lys
            580                 585                 590

Gly Leu Trp Thr Gly Thr Pro Phe Phe Tyr Pro Gly Met Asn Ser Ile
        595                 600                 605

Leu Val Tyr Val Gly His Glu Val Phe Glu Asn Tyr Phe Pro Phe Gln
    610                 615                 620

Trp Lys Leu Lys Asp Asn Gln Ser His Lys Glu His Leu Thr Gln Asn
625                 630                 635                 640

Ile Val Ala Thr Ala Leu Trp Val Leu Ile Ala Tyr Ile Leu Tyr Arg
                645                 650                 655

Lys Lys Ile Phe Trp Lys Ile
            660
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<210> SEQ ID NO 21
<211> LENGTH: 743
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

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Met Glu Ala Val Ala Val Ala Ala Ala Val Gly Val Leu Leu Leu Ala
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1               5                   10                  15

Gly Ala Gly Gly Ala Ala Gly Asp Glu Ala Arg Glu Ala Ala Ala Val
            20                  25                  30

Arg Ala Leu Val Ala Arg Leu Leu Gly Pro Gly Pro Ala Ala Asp Phe
        35                  40                  45

Ser Val Ser Val Glu Arg Ala Leu Ala Ala Lys Pro Gly Leu Asp Thr
    50                  55                  60

Tyr Ser Leu Gly Gly Gly Gly Ala Ala Arg Val Arg Val Arg Gly Ser
65                  70                  75                  80

Thr Gly Val Ala Ala Ala Ala Gly Leu His Arg Tyr Leu Arg Asp Phe
                85                  90                  95

Cys Gly Cys His Val Ala Trp Ser Gly Ser Gln Leu Arg Leu Pro Arg
            100                 105                 110

Pro Leu Pro Ala Val Pro Gly Glu Leu Thr Glu Ala Thr Pro Asn Arg
        115                 120                 125

Tyr Arg Tyr Tyr Gln Asn Val Cys Thr Gln Ser Tyr Ser Phe Val Trp
    130                 135                 140

Trp Asp Trp Ala Arg Trp Glu Arg Glu Ile Asp Trp Met Ala Leu Asn
145                 150                 155                 160

Gly Ile Asn Leu Ala Leu Ala Trp Ser Gly Gln Glu Ala Ile Trp Gln
                165                 170                 175

Arg Val Tyr Leu Ala Leu Gly Leu Thr Gln Ala Glu Ile Asn Glu Phe
            180                 185                 190

Phe Thr Gly Pro Ala Phe Leu Ala Trp Gly Arg Met Gly Asn Leu His
        195                 200                 205

Thr Trp Asp Gly Pro Leu Pro Pro Ser Trp His Ile Lys Gln Leu Tyr
    210                 215                 220

Leu Gln His Arg Val Leu Asp Gln Met Arg Ser Phe Gly Met Thr Pro
225                 230                 235                 240

Val Leu Pro Ala Phe Ala Gly His Val Pro Glu Ala Val Thr Arg Val
                245                 250                 255

Phe Pro Gln Val Asn Val Thr Lys Met Gly Ser Trp Gly His Phe Asn
            260                 265                 270

Cys Ser Tyr Ser Cys Ser Phe Leu Leu Ala Pro Glu Asp Pro Ile Phe
        275                 280                 285

Pro Ile Ile Gly Ser Leu Phe Leu Arg Glu Leu Ile Lys Glu Phe Gly
    290                 295                 300

Thr Asp His Ile Tyr Gly Ala Asp Thr Phe Asn Glu Met Gln Pro Pro
305                 310                 315                 320

Ser Ser Glu Pro Ser Tyr Leu Ala Ala Ala Thr Thr Ala Val Tyr Glu
                325                 330                 335

Ala Met Thr Ala Val Asp Thr Glu Ala Val Trp Leu Leu Gln Gly Trp
            340                 345                 350

Leu Phe Gln His Gln Pro Gln Phe Trp Gly Pro Ala Gln Ile Arg Ala
        355                 360                 365

Val Leu Gly Ala Val Pro Arg Gly Arg Leu Leu Val Leu Asp Leu Phe
    370                 375                 380

Ala Glu Ser Gln Pro Val Tyr Thr Arg Thr Ala Ser Phe Gln Gly Gln
385                 390                 395                 400

Pro Phe Ile Trp Cys Met Leu His Asn Phe Gly Gly Asn His Gly Leu
                405                 410                 415
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Phe Gly Ala Leu Glu Ala Val Asn Gly Gly Pro Glu Ala Ala Arg Leu
            420                 425                 430

Phe Pro Asn Ser Thr Met Val Gly Thr Gly Met Ala Pro Glu Gly Ile
        435                 440                 445

Ser Gln Asn Glu Val Val Tyr Ser Leu Met Ala Glu Leu Gly Trp Arg
    450                 455                 460

Lys Asp Pro Val Pro Asp Leu Ala Ala Trp Val Thr Ser Phe Ala Ala
465                 470                 475                 480

Arg Arg Tyr Gly Val Ser His Pro Asp Ala Gly Ala Ala Trp Arg Leu
                485                 490                 495

Leu Leu Arg Ser Val Tyr Asn Cys Ser Gly Glu Ala Cys Arg Gly His
            500                 505                 510

Asn Arg Ser Pro Leu Val Arg Arg Pro Ser Leu Gln Met Asn Thr Ser
        515                 520                 525

Ile Trp Tyr Asn Arg Ser Asp Val Phe Glu Ala Trp Arg Leu Leu Leu
    530                 535                 540

Thr Ser Ala Pro Ser Leu Ala Thr Ser Pro Ala Phe Arg Tyr Asp Leu
545                 550                 555                 560

Leu Asp Leu Thr Arg Gln Ala Val Gln Glu Leu Val Ser Leu Tyr Tyr
                565                 570                 575

Glu Glu Ala Arg Ser Ala Tyr Leu Ser Lys Glu Leu Ala Ser Leu Leu
            580                 585                 590

Arg Ala Gly Gly Val Leu Ala Tyr Glu Leu Leu Pro Ala Leu Asp Glu
        595                 600                 605

Val Leu Ala Ser Asp Ser Arg Phe Leu Leu Gly Ser Trp Leu Glu Gln
    610                 615                 620

Ala Arg Ala Ala Ala Val Ser Glu Ala Glu Ala Asp Phe Tyr Glu Gln
625                 630                 635                 640

Asn Ser Arg Tyr Gln Leu Thr Leu Trp Gly Pro Glu Gly Asn Ile Leu
                645                 650                 655

Asp Tyr Ala Asn Lys Gln Leu Ala Gly Leu Val Ala Asn Tyr Tyr Thr
            660                 665                 670

Pro Arg Trp Arg Leu Phe Leu Glu Ala Leu Val Asp Ser Val Ala Gln
        675                 680                 685

Gly Ile Pro Phe Gln Gln His Gln Phe Asp Lys Asn Val Phe Gln Leu
    690                 695                 700

Glu Gln Ala Phe Val Leu Ser Lys Gln Arg Tyr Pro Ser Gln Pro Arg
705                 710                 715                 720

Gly Asp Thr Val Asp Leu Ala Lys Lys Ile Phe Leu Lys Tyr Tyr Pro
                725                 730                 735

Arg Trp Val Ala Gly Ser Trp
            740


<210> SEQ ID NO 22
<211> LENGTH: 663
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Met Thr Gly Ala Arg Ala Ser Ala Ala Glu Gln Arg Arg Ala Gly Arg
1               5                   10                  15

Ser Gly Gln Ala Arg Ala Ala Glu Arg Ala Ala Gly Met Ser Gly Ala
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            20                  25                  30

Gly Arg Ala Leu Ala Ala Leu Leu Leu Ala Ala Ser Val Leu Ser Ala
        35                  40                  45

Ala Leu Leu Ala Pro Gly Gly Ser Ser Gly Arg Asp Ala Gln Ala Ala
    50                  55                  60

Pro Pro Arg Asp Leu Asp Lys Lys Arg His Ala Glu Leu Lys Met Asp
65                  70                  75                  80

Gln Ala Leu Leu Leu Ile His Asn Glu Leu Leu Trp Thr Asn Leu Thr
                85                  90                  95

Val Tyr Trp Lys Ser Glu Cys Cys Tyr His Cys Leu Phe Gln Val Leu
            100                 105                 110

Val Asn Val Pro Gln Ser Pro Lys Ala Gly Lys Pro Ser Ala Ala Ala
        115                 120                 125

Ala Ser Val Ser Thr Gln His Gly Ser Ile Leu Gln Leu Asn Asp Thr
    130                 135                 140

Leu Glu Glu Lys Glu Val Cys Arg Leu Glu Tyr Arg Phe Gly Glu Phe
145                 150                 155                 160

Gly Asn Tyr Ser Leu Leu Val Lys Asn Ile His Asn Gly Val Ser Glu
                165                 170                 175

Ile Ala Cys Asp Leu Ala Val Asn Glu Asp Pro Val Asp Ser Asn Leu
            180                 185                 190

Pro Val Ser Ile Ala Phe Leu Ile Gly Leu Ala Val Ile Ile Val Ile
        195                 200                 205

Ser Phe Leu Arg Leu Leu Leu Ser Leu Asp Asp Phe Asn Asn Trp Ile
    210                 215                 220

Ser Lys Ala Ile Ser Ser Arg Glu Thr Asp Arg Leu Ile Asn Ser Glu
225                 230                 235                 240

Leu Gly Ser Pro Ser Arg Thr Asp Pro Leu Asp Gly Asp Val Gln Pro
                245                 250                 255

Ala Thr Trp Arg Leu Ser Ala Leu Pro Pro Arg Leu Arg Ser Val Asp
            260                 265                 270

Thr Phe Arg Gly Ile Ala Leu Ile Leu Met Val Phe Val Asn Tyr Gly
        275                 280                 285

Gly Gly Lys Tyr Trp Tyr Phe Lys His Ala Ser Trp Asn Gly Leu Thr
    290                 295                 300

Val Ala Asp Leu Val Phe Pro Trp Phe Val Phe Ile Met Gly Ser Ser
305                 310                 315                 320

Ile Phe Leu Ser Met Thr Ser Ile Leu Gln Arg Gly Cys Ser Lys Phe
                325                 330                 335

Arg Leu Leu Gly Lys Ile Ala Trp Arg Ser Phe Leu Leu Ile Cys Ile
            340                 345                 350

Gly Ile Ile Ile Val Asn Pro Asn Tyr Cys Leu Gly Pro Leu Ser Trp
        355                 360                 365

Asp Lys Val Arg Ile Pro Gly Val Leu Gln Arg Leu Gly Val Thr Tyr
    370                 375                 380

Phe Val Val Ala Val Leu Glu Leu Leu Phe Ala Lys Pro Val Pro Glu
385                 390                 395                 400

His Cys Ala Ser Glu Arg Ser Cys Leu Ser Leu Arg Asp Ile Thr Ser
                405                 410                 415

Ser Trp Pro Gln Trp Leu Leu Ile Leu Val Leu Glu Gly Leu Trp Leu
            420                 425                 430
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Gly Leu Thr Phe Leu Leu Pro Val Pro Gly Cys Pro Thr Gly Tyr Leu
435 440 445

Gly Pro Gly Gly Ile Gly Asp Phe Gly Lys Tyr Pro Asn Cys Thr Gly
450 455 460

Gly Ala Ala Gly Tyr Ile Asp Arg Leu Leu Leu Gly Asp Asp His Leu
465 470 475 480

Tyr Gln His Pro Ser Ser Ala Val Leu Tyr His Thr Glu Val Ala Tyr
485 490 495

Asp Pro Glu Gly Ile Leu Gly Thr Ile Asn Ser Ile Val Met Ala Phe
500 505 510

Leu Gly Val Gln Ala Gly Lys Ile Leu Leu Tyr Tyr Lys Ala Arg Thr
515 520 525

Lys Asp Ile Leu Ile Arg Phe Thr Ala Trp Cys Cys Ile Leu Gly Leu
530 535 540

Ile Ser Val Ala Leu Thr Lys Val Ser Glu Asn Glu Gly Phe Ile Pro
545 550 555 560

Val Asn Lys Asn Leu Trp Ser Leu Ser Tyr Val Thr Thr Leu Ser Ser
565 570 575

Phe Ala Phe Phe Ile Leu Leu Val Leu Tyr Pro Val Val Asp Val Lys
580 585 590

Gly Leu Trp Thr Gly Thr Pro Phe Phe Tyr Pro Gly Met Asn Ser Ile
595 600 605

Leu Val Tyr Val Gly His Glu Val Phe Glu Asn Tyr Phe Pro Phe Gln
610 615 620

Trp Lys Leu Lys Asp Asn Gln Ser His Lys Glu His Leu Thr Gln Asn
625 630 635 640

Ile Val Ala Thr Ala Leu Trp Val Leu Ile Ala Tyr Ile Leu Tyr Arg
645 650 655

Lys Lys Ile Phe Trp Lys Ile
660

<210> SEQ ID NO 23
<211> LENGTH: 552
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Met Arg Leu Leu Pro Leu Ala Pro Gly Arg Leu Arg Arg Gly Ser Pro
1 5 10 15

Arg His Leu Pro Ser Cys Ser Pro Ala Leu Leu Leu Leu Val Leu Gly
20 25 30

Gly Cys Leu Gly Val Phe Gly Val Ala Ala Gly Thr Arg Arg Pro Asn
35 40 45

Val Val Leu Leu Leu Thr Asp Asp Gln Asp Glu Val Leu Gly Gly Met
50 55 60

Thr Pro Leu Lys Lys Thr Lys Ala Leu Ile Gly Glu Met Gly Met Thr
65 70 75 80

Phe Ser Ser Ala Tyr Val Pro Ser Ala Leu Cys Cys Pro Ser Arg Ala
85 90 95

Ser Ile Leu Thr Gly Lys Tyr Pro His Asn His His Val Val Asn Asn
100 105 110

Thr Leu Glu Gly Asn Cys Ser Ser Lys Ser Trp Gln Lys Ile Gln Glu

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        115                 120                 125

Pro Asn Thr Phe Pro Ala Ile Leu Arg Ser Met Cys Gly Tyr Gln Thr
    130                 135                 140

Phe Phe Ala Gly Lys Tyr Leu Asn Glu Tyr Gly Ala Pro Asp Ala Gly
145                 150                 155                 160

Gly Leu Glu His Val Pro Leu Gly Trp Ser Tyr Trp Tyr Ala Leu Glu
                165                 170                 175

Lys Asn Ser Lys Tyr Tyr Asn Tyr Thr Leu Ser Ile Asn Gly Lys Ala
            180                 185                 190

Arg Lys His Gly Glu Asn Tyr Ser Val Asp Tyr Leu Thr Asp Val Leu
        195                 200                 205

Ala Asn Val Ser Leu Asp Phe Leu Asp Tyr Lys Ser Asn Phe Glu Pro
    210                 215                 220

Phe Phe Met Met Ile Ala Thr Pro Ala Pro His Ser Pro Trp Thr Ala
225                 230                 235                 240

Ala Pro Gln Tyr Gln Lys Ala Phe Gln Asn Val Phe Ala Pro Arg Asn
                245                 250                 255

Lys Asn Phe Asn Ile His Gly Thr Asn Lys His Trp Leu Ile Arg Gln
            260                 265                 270

Ala Lys Thr Pro Met Thr Asn Ser Ser Ile Gln Phe Leu Asp Asn Ala
        275                 280                 285

Phe Arg Lys Arg Trp Gln Thr Leu Leu Ser Val Asp Asp Leu Val Glu
    290                 295                 300

Lys Leu Val Lys Arg Leu Glu Phe Thr Gly Glu Leu Asn Asn Thr Tyr
305                 310                 315                 320

Ile Phe Tyr Thr Ser Asp Asn Gly Tyr His Thr Gly Gln Phe Ser Leu
                325                 330                 335

Pro Ile Asp Lys Arg Gln Leu Tyr Glu Phe Asp Ile Lys Val Pro Leu
            340                 345                 350

Leu Val Arg Gly Pro Gly Ile Lys Pro Asn Gln Thr Ser Lys Met Leu
        355                 360                 365

Val Ala Asn Ile Asp Leu Gly Pro Thr Ile Leu Asp Ile Ala Gly Tyr
    370                 375                 380

Asp Leu Asn Lys Thr Gln Met Asp Gly Met Ser Leu Leu Pro Ile Leu
385                 390                 395                 400

Arg Gly Ala Ser Asn Leu Thr Trp Arg Ser Asp Val Leu Val Glu Tyr
                405                 410                 415

Gln Gly Glu Gly Arg Asn Val Thr Asp Pro Thr Cys Pro Ser Leu Ser
            420                 425                 430

Pro Gly Val Ser Gln Cys Phe Pro Asp Cys Val Cys Glu Asp Ala Tyr
        435                 440                 445

Asn Asn Thr Tyr Ala Cys Val Arg Thr Met Ser Ala Leu Trp Asn Leu
    450                 455                 460

Gln Tyr Cys Glu Phe Asp Asp Gln Glu Val Phe Val Glu Val Tyr Asn
465                 470                 475                 480

Leu Thr Ala Asp Pro Asp Gln Ile Thr Asn Ile Ala Lys Thr Ile Asp
                485                 490                 495

Pro Glu Leu Leu Gly Lys Met Asn Tyr Arg Leu Met Met Leu Gln Ser
            500                 505                 510

Cys Ser Gly Pro Thr Cys Arg Thr Pro Gly Val Phe Asp Pro Gly Tyr
        515                 520                 525
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Arg Phe Asp Pro Arg Leu Met Phe Ser Asn Arg Gly Ser Val Arg Thr
530 535 540

Arg Arg Phe Ser Lys His Leu Leu
545 550

<210> SEQ ID NO 24
<211> LENGTH: 411
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Met Leu Leu Lys Thr Val Leu Leu Leu Gly His Val Ala Gln Val Leu
1 5 10 15

Met Leu Asp Asn Gly Leu Leu Gln Thr Pro Pro Met Gly Trp Leu Ala
20 25 30

Trp Glu Arg Phe Arg Cys Asn Ile Asn Cys Asp Glu Asp Pro Lys Asn
35 40 45

Cys Ile Ser Glu Gln Leu Phe Met Glu Met Ala Asp Arg Met Ala Gln
50 55 60

Asp Gly Trp Arg Asp Met Gly Tyr Thr Tyr Leu Asn Ile Asp Asp Cys
65 70 75 80

Trp Ile Gly Gly Arg Asp Ala Ser Gly Arg Leu Met Pro Asp Pro Lys
85 90 95

Arg Phe Pro His Gly Ile Pro Phe Leu Ala Asp Tyr Val His Ser Leu
100 105 110

Gly Leu Lys Leu Gly Ile Tyr Ala Asp Met Gly Asn Phe Thr Cys Met
115 120 125

Gly Tyr Pro Gly Thr Thr Leu Asp Lys Val Val Gln Asp Ala Gln Thr
130 135 140

Phe Ala Glu Trp Lys Val Asp Met Leu Lys Leu Asp Gly Cys Phe Ser
145 150 155 160

Thr Pro Glu Glu Arg Ala Gln Gly Tyr Pro Lys Met Ala Ala Ala Leu
165 170 175

Asn Ala Thr Gly Arg Pro Ile Ala Phe Ser Cys Ser Trp Pro Ala Tyr
180 185 190

Glu Gly Gly Leu Pro Pro Arg Val Asn Tyr Ser Leu Leu Ala Asp Ile
195 200 205

Cys Asn Leu Trp Arg Asn Tyr Asp Asp Ile Gln Asp Ser Trp Trp Ser
210 215 220

Val Leu Ser Ile Leu Asn Trp Phe Val Glu His Gln Asp Ile Leu Gln
225 230 235 240

Pro Val Ala Gly Pro Gly His Trp Asn Asp Pro Asp Met Leu Leu Ile
245 250 255

Gly Asn Phe Gly Leu Ser Leu Glu Gln Ser Arg Ala Gln Met Ala Leu
260 265 270

Trp Thr Val Leu Ala Ala Pro Leu Leu Met Ser Thr Asp Leu Arg Thr
275 280 285

Ile Ser Ala Gln Asn Met Asp Ile Leu Gln Asn Pro Leu Met Ile Lys
290 295 300

Ile Asn Gln Asp Pro Leu Gly Ile Gln Gly Arg Arg Ile His Lys Glu
305 310 315 320

Lys Ser Leu Ile Glu Val Tyr Met Arg Pro Leu Ser Asn Lys Ala Ser

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```
                325                 330                 335

Ala Leu Val Phe Phe Ser Cys Arg Thr Asp Met Pro Tyr Arg Tyr His
            340                 345                 350

Ser Ser Leu Gly Gln Leu Asn Phe Thr Gly Ser Val Ile Tyr Glu Ala
        355                 360                 365

Gln Asp Val Tyr Ser Gly Asp Ile Ile Ser Gly Leu Arg Asp Glu Thr
    370                 375                 380

Asn Phe Thr Val Ile Ile Asn Pro Ser Gly Val Val Met Trp Tyr Leu
385                 390                 395                 400

Tyr Pro Ile Lys Asn Leu Glu Met Ser Gln Gln
                405                 410
```

<210> SEQ ID NO 25
<211> LENGTH: 415
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

```
Met Thr Gly Glu Arg Pro Ser Thr Ala Leu Pro Asp Arg Arg Trp Gly
1               5                   10                  15

Pro Arg Ile Leu Gly Phe Trp Gly Gly Cys Arg Val Trp Val Phe Ala
            20                  25                  30

Ala Ile Phe Leu Leu Leu Ser Leu Ala Ala Ser Trp Ser Lys Ala Glu
        35                  40                  45

Asn Asp Phe Gly Leu Val Gln Pro Leu Val Thr Met Glu Gln Leu Leu
    50                  55                  60

Trp Val Ser Gly Arg Gln Ile Gly Ser Val Asp Thr Phe Arg Ile Pro
65                  70                  75                  80

Leu Ile Thr Ala Thr Pro Arg Gly Thr Leu Leu Ala Phe Ala Glu Ala
                85                  90                  95

Arg Lys Met Ser Ser Ser Asp Glu Gly Ala Lys Phe Ile Ala Leu Arg
            100                 105                 110

Arg Ser Met Asp Gln Gly Ser Thr Trp Ser Pro Thr Ala Phe Ile Val
        115                 120                 125

Asn Asp Gly Asp Val Pro Asp Gly Leu Asn Leu Gly Ala Val Val Ser
    130                 135                 140

Asp Val Glu Thr Gly Val Val Phe Leu Phe Tyr Ser Leu Cys Ala His
145                 150                 155                 160

Lys Ala Gly Cys Gln Val Ala Ser Thr Met Leu Val Trp Ser Lys Asp
                165                 170                 175

Asp Gly Val Ser Trp Ser Thr Pro Arg Asn Leu Ser Leu Asp Ile Gly
            180                 185                 190

Thr Glu Val Phe Ala Pro Gly Pro Gly Ser Gly Ile Gln Lys Gln Arg
        195                 200                 205

Glu Pro Arg Lys Gly Arg Leu Ile Val Cys Gly His Gly Thr Leu Glu
    210                 215                 220

Arg Asp Gly Val Phe Cys Leu Leu Ser Asp Asp His Gly Ala Ser Trp
225                 230                 235                 240

Arg Tyr Gly Ser Gly Val Ser Gly Ile Pro Tyr Gly Gln Pro Lys Gln
                245                 250                 255

Glu Asn Asp Phe Asn Pro Asp Glu Cys Gln Pro Tyr Glu Leu Pro Asp
            260                 265                 270
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Gly Ser Val Val Ile Asn Ala Arg Asn Gln Asn Asn Tyr His Cys His
        275                 280                 285

Cys Arg Ile Val Leu Arg Ser Tyr Asp Ala Cys Asp Thr Leu Arg Pro
    290                 295                 300

Arg Asp Val Thr Phe Asp Pro Glu Leu Val Asp Pro Val Val Ala Ala
305                 310                 315                 320

Gly Ala Val Val Thr Ser Ser Gly Ile Val Phe Phe Ser Asn Pro Ala
                325                 330                 335

His Pro Glu Phe Arg Val Asn Leu Thr Leu Arg Trp Ser Phe Ser Asn
            340                 345                 350

Gly Thr Ser Trp Arg Lys Glu Thr Val Gln Leu Trp Pro Gly Pro Ser
        355                 360                 365

Gly Tyr Ser Ser Leu Ala Thr Leu Glu Gly Ser Met Asp Gly Glu Glu
    370                 375                 380

Gln Ala Pro Gln Leu Tyr Val Leu Tyr Glu Lys Gly Arg Asn His Tyr
385                 390                 395                 400

Thr Glu Ser Ile Ser Val Ala Lys Ile Ser Val Tyr Gly Thr Leu
                405                 410                 415


<210> SEQ ID NO 26
<211> LENGTH: 651
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Met Ala Arg Gly Ser Ala Val Ala Trp Ala Ala Leu Gly Pro Leu Leu
1               5                   10                  15

Trp Gly Cys Ala Leu Gly Leu Gln Gly Gly Met Leu Tyr Pro Gln Glu
            20                  25                  30

Ser Pro Ser Arg Glu Cys Lys Glu Leu Asp Gly Leu Trp Ser Phe Arg
        35                  40                  45

Ala Asp Phe Ser Asp Asn Arg Arg Arg Gly Phe Glu Glu Gln Trp Tyr
    50                  55                  60

Arg Arg Pro Leu Trp Glu Ser Gly Pro Thr Val Asp Met Pro Val Pro
65                  70                  75                  80

Ser Ser Phe Asn Asp Ile Ser Gln Asp Trp Arg Leu Arg His Phe Val
                85                  90                  95

Gly Trp Val Trp Tyr Glu Arg Glu Val Ile Leu Pro Glu Arg Trp Thr
            100                 105                 110

Gln Asp Leu Arg Thr Arg Val Val Leu Arg Ile Gly Ser Ala His Ser
        115                 120                 125

Tyr Ala Ile Val Trp Val Asn Gly Val Asp Thr Leu Glu His Glu Gly
    130                 135                 140

Gly Tyr Leu Pro Phe Glu Ala Asp Ile Ser Asn Leu Val Gln Val Gly
145                 150                 155                 160

Pro Leu Pro Ser Arg Leu Arg Ile Thr Ile Ala Ile Asn Asn Thr Leu
                165                 170                 175

Thr Pro Thr Thr Leu Pro Pro Gly Thr Ile Gln Tyr Leu Thr Asp Thr
            180                 185                 190

Ser Lys Tyr Pro Lys Gly Tyr Phe Val Gln Asn Thr Tyr Phe Asp Phe
        195                 200                 205

Phe Asn Tyr Ala Gly Leu Gln Arg Ser Val Leu Leu Tyr Thr Thr Pro
    210                 215                 220
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Thr Thr Tyr Ile Asp Asp Ile Thr Val Thr Thr Ser Val Glu Gln Asp
225                 230                 235                 240

Ser Gly Leu Val Asn Tyr Gln Ile Ser Val Lys Gly Ser Asn Leu Phe
                245                 250                 255

Lys Leu Glu Val Arg Leu Leu Asp Ala Glu Asn Lys Val Val Ala Asn
            260                 265                 270

Gly Thr Gly Thr Gln Gly Gln Leu Lys Val Pro Gly Val Ser Leu Trp
        275                 280                 285

Trp Pro Tyr Leu Met His Glu Arg Pro Ala Tyr Leu Tyr Ser Leu Glu
    290                 295                 300

Val Gln Leu Thr Ala Gln Thr Ser Leu Gly Pro Val Ser Asp Phe Tyr
305                 310                 315                 320

Thr Leu Pro Val Gly Ile Arg Thr Val Ala Val Thr Lys Ser Gln Phe
                325                 330                 335

Leu Ile Asn Gly Lys Pro Phe Tyr Phe His Gly Val Asn Lys His Glu
            340                 345                 350

Asp Ala Asp Ile Arg Gly Lys Gly Phe Asp Trp Pro Leu Leu Val Lys
        355                 360                 365

Asp Phe Asn Leu Leu Arg Trp Leu Gly Ala Asn Ala Phe Arg Thr Ser
    370                 375                 380

His Tyr Pro Tyr Ala Glu Glu Val Met Gln Met Cys Asp Arg Tyr Gly
385                 390                 395                 400

Ile Val Val Ile Asp Glu Cys Pro Gly Val Gly Leu Ala Leu Pro Gln
                405                 410                 415

Phe Phe Asn Asn Val Ser Leu His His His Met Gln Val Met Glu Glu
            420                 425                 430

Val Val Arg Arg Asp Lys Asn His Pro Ala Val Val Met Trp Ser Val
        435                 440                 445

Ala Asn Glu Pro Ala Ser His Leu Glu Ser Ala Gly Tyr Tyr Leu Lys
    450                 455                 460

Met Val Ile Ala His Thr Lys Ser Leu Asp Pro Ser Arg Ser Val Thr
465                 470                 475                 480

Phe Val Ser Asn Ser Asn Tyr Ala Ala Asp Lys Gly Ala Pro Tyr Val
                485                 490                 495

Asp Val Ile Cys Leu Asn Ser Tyr Tyr Ser Trp Tyr His Asp Tyr Gly
            500                 505                 510

His Leu Glu Leu Ile Gln Leu Gln Leu Ala Thr Gln Phe Glu Asn Trp
        515                 520                 525

Tyr Lys Lys Tyr Gln Lys Pro Ile Ile Gln Ser Glu Tyr Gly Ala Glu
    530                 535                 540

Thr Ile Ala Gly Phe His Gln Asp Pro Pro Leu Met Phe Thr Glu Glu
545                 550                 555                 560

Tyr Gln Lys Ser Leu Leu Glu Gln Tyr His Leu Gly Leu Asp Gln Lys
                565                 570                 575

Arg Arg Lys Tyr Val Val Gly Glu Leu Ile Trp Asn Phe Ala Asp Phe
            580                 585                 590

Met Thr Glu Gln Ser Pro Thr Arg Val Leu Gly Asn Lys Lys Gly Ile
        595                 600                 605

Phe Thr Arg Gln Arg Gln Pro Lys Ser Ala Ala Phe Leu Leu Arg Glu
    610                 615                 620
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Arg Tyr Trp Lys Ile Ala Asn Glu Thr Arg Tyr Pro His Ser Val Ala
625 630 635 640

Lys Ser Gln Cys Leu Glu Asn Ser Pro Phe Thr
645 650

<210> SEQ ID NO 27
<211> LENGTH: 556
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

Met Glu Leu Cys Gly Leu Gly Leu Pro Arg Pro Pro Met Leu Leu Ala
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Leu Leu Leu Ala Thr Leu Leu Ala Ala Met Leu Ala Leu Leu Thr Gln
20 25 30

Val Ala Leu Val Val Gln Val Ala Glu Ala Ala Arg Ala Pro Ser Val
35 40 45

Ser Ala Lys Pro Gly Pro Ala Leu Trp Pro Leu Pro Leu Leu Val Lys
50 55 60

Met Thr Pro Asn Leu Leu His Leu Ala Pro Glu Asn Phe Tyr Ile Ser
65 70 75 80

His Ser Pro Asn Ser Thr Ala Gly Pro Ser Cys Thr Leu Leu Glu Glu
85 90 95

Ala Phe Arg Arg Tyr His Gly Tyr Ile Phe Gly Phe Tyr Lys Trp His
100 105 110

His Glu Pro Ala Glu Phe Gln Ala Lys Thr Gln Val Gln Gln Leu Leu
115 120 125

Val Ser Ile Thr Leu Gln Ser Glu Cys Asp Ala Phe Pro Asn Ile Ser
130 135 140

Ser Asp Glu Ser Tyr Thr Leu Leu Val Lys Glu Pro Val Ala Val Leu
145 150 155 160

Lys Ala Asn Arg Val Trp Gly Ala Leu Arg Gly Leu Glu Thr Phe Ser
165 170 175

Gln Leu Val Tyr Gln Asp Ser Tyr Gly Thr Phe Thr Ile Asn Glu Ser
180 185 190

Thr Ile Ile Asp Ser Pro Arg Phe Ser His Arg Gly Ile Leu Ile Asp
195 200 205

Thr Ser Arg His Tyr Leu Pro Val Lys Ile Ile Leu Lys Thr Leu Asp
210 215 220

Ala Met Ala Phe Asn Lys Phe Asn Val Leu His Trp His Ile Val Asp
225 230 235 240

Asp Gln Ser Phe Pro Tyr Gln Ser Ile Thr Phe Pro Glu Leu Ser Asn
245 250 255

Lys Gly Ser Tyr Ser Leu Ser His Val Tyr Thr Pro Asn Asp Val Arg
260 265 270

Met Val Ile Glu Tyr Ala Arg Leu Arg Gly Ile Arg Val Leu Pro Glu
275 280 285

Phe Asp Thr Pro Gly His Thr Leu Ser Trp Gly Lys Gly Gln Lys Asp
290 295 300

Leu Leu Thr Pro Cys Tyr Ser Arg Gln Asn Lys Leu Asp Ser Phe Gly
305 310 315 320

Pro Ile Asn Pro Thr Leu Asn Thr Thr Tyr Ser Phe Leu Thr Thr Phe
325 330 335

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Phe Lys Glu Ile Ser Glu Val Phe Pro Asp Gln Phe Ile His Leu Gly
            340                 345                 350

Gly Asp Glu Val Glu Phe Lys Cys Trp Glu Ser Asn Pro Lys Ile Gln
        355                 360                 365

Asp Phe Met Arg Gln Lys Gly Phe Gly Thr Asp Phe Lys Lys Leu Glu
    370                 375                 380

Ser Phe Tyr Ile Gln Lys Val Leu Asp Ile Ile Ala Thr Ile Asn Lys
385                 390                 395                 400

Gly Ser Ile Val Trp Gln Glu Val Phe Asp Asp Lys Ala Lys Leu Ala
                405                 410                 415

Pro Gly Thr Ile Val Glu Val Trp Lys Asp Ser Ala Tyr Pro Glu Glu
            420                 425                 430

Leu Ser Arg Val Thr Ala Ser Gly Phe Pro Val Ile Leu Ser Ala Pro
        435                 440                 445

Trp Tyr Leu Asp Leu Ile Ser Tyr Gly Gln Asp Trp Arg Lys Tyr Tyr
    450                 455                 460

Lys Val Glu Pro Leu Asp Phe Gly Gly Thr Gln Lys Gln Lys Gln Leu
465                 470                 475                 480

Phe Ile Gly Gly Glu Ala Cys Leu Trp Gly Glu Tyr Val Asp Ala Thr
                485                 490                 495

Asn Leu Thr Pro Arg Leu Trp Pro Arg Ala Ser Ala Val Gly Glu Arg
            500                 505                 510

Leu Trp Ser Ser Lys Asp Val Arg Asp Met Asp Asp Ala Tyr Asp Arg
        515                 520                 525

Leu Thr Arg His Arg Cys Arg Met Val Glu Arg Gly Ile Ala Ala Gln
    530                 535                 540

Pro Leu Tyr Ala Gly Tyr Cys Asn His Glu Asn Met
545                 550                 555
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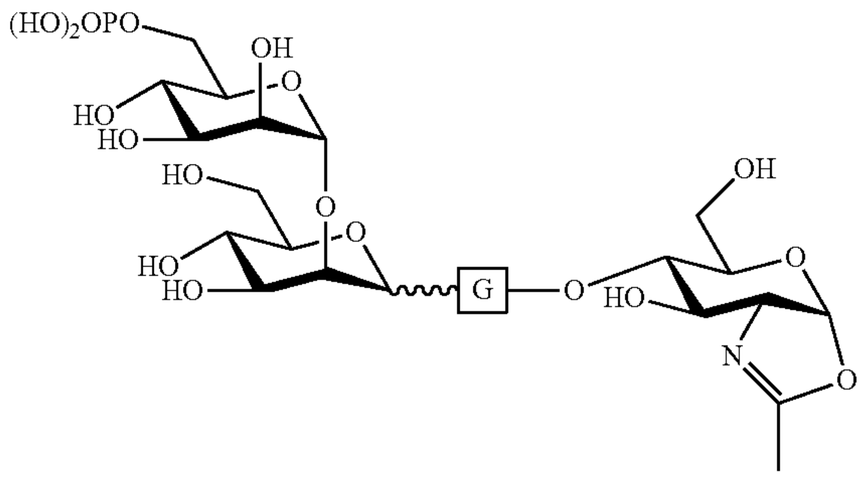
1. A compound of Formula (I), or a salt thereof,

(I)

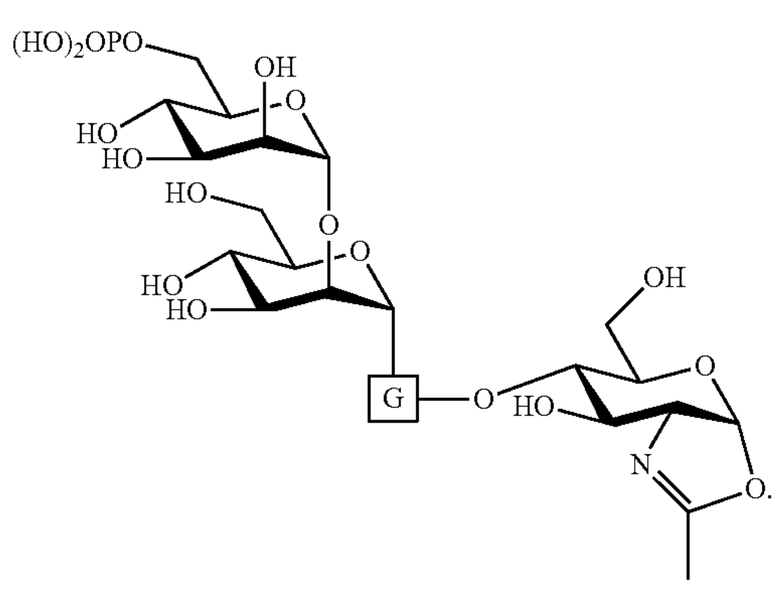


wherein G is sugar moiety or linker.

2. (canceled)

3. The compound of claim **1**, having a structure of Formula (I-a), or a salt thereof

(I-a)



4. The compound of claim **1**, or a salt thereof, wherein G is a sugar moiety, optionally wherein G is a monosaccharide moiety, a disaccharide moiety, a trisaccharide moiety, or a tetrasaccharide moiety.

5. (canceled)

6. (canceled)

7. (canceled)

8. A method for remodeling a glycoprotein, comprising:

(a) contacting the glycoprotein with an endoglycosidase selected from the group consisting of wild type Endo A, wild type Endo F3, wild type Endo-CC, and a combination of, thereby producing a deglycosylated intermediate comprising a N-acetylglucosamine (GlcNAc) or core-fucosylated N-acetylglucosamine (Fuca1, 6GlcNAc) acceptor from the glycoprotein by a deglycosylation activity of the endoglycosidase to produce a deglycosylated intermediate; and

(b) contacting a glycan oxazoline comprising a mannose-6-phosphate (M6P) moiety with the deglycosylated intermediate in the presence of the endoglycosidase, thereby attaching the glycan oxazoline to the N-acetylglucosamine (GlcNAc) or core-fucosylated N-acetylglucosamine (Fuca1,6GlcNAc) acceptor by a transglycosylation activity of the endoglycosidase, thereby producing a remodeled glycoprotein,

wherein (a) and (b) are carried out in a one-pot reaction.

9. The method of claim **8**, wherein the glycoprotein is a lysosomal enzyme.

10. The method of claim **8**, wherein the endoglycosidase is wild type Endo A, optionally wherein the wild type Endo A removes high-mannose and hybrid type glycans from the lysosomal enzyme without affecting complex-type glycans.

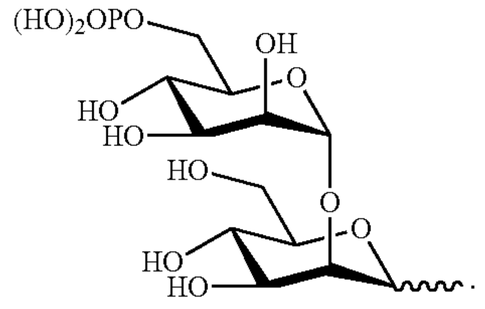
11. The method of claim **8**, wherein the endoglycosidase is wild type Endo F3, optionally wherein the wild type Endo F3 removes core-fucosylated complex-type glycans from the lysosomal enzyme without affecting high-mannose or hybrid type glycans.

12. The method of claim **8**, wherein the endoglycosidase is wild type Endo-CC, optionally wherein the wild type Endo-CC removes high-mannose type and biantennary complex type glycans from the lysosomal enzyme without affecting core-fucosylated complex-type glycans or higher branched complex type glycans.

13. The method of claim **8**, wherein the endoglycosidase is a combination of the wild type Endo A and the wild type Endo F3.

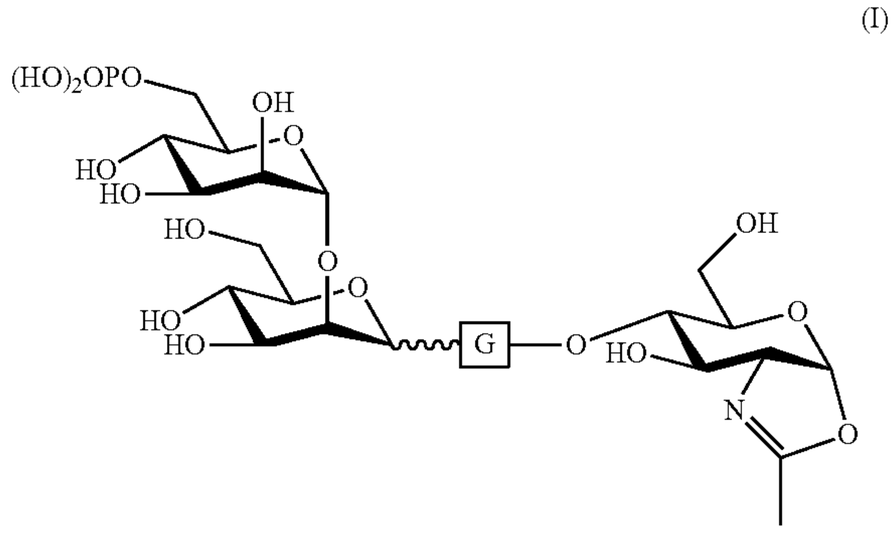
14. The method of claim **8**, wherein the lysosomal enzyme is selected from the group consisting of α-galactosidase A, acid ceramidase, acid α-L-fucosidase, acid β-glucosidase, acid β-galactosidase, iduronate-2-sulfatase, α-L-iduronidase, galactocerebrosidase, acid α-mannosidase, acid β-mannosidase, arylsulfatase B, arylsulfatase A, N-acetylgalactosamine-6-sulfate sulfatase (N-acetylgalactosamine-6-sulfatase, or galactose-6-sulfatase), acid β-galactosidase, acid sphingomyelinase, acid α-glucosidase (α-glucosidase), β-hexosaminidase B, heparan N-sulfatase, α-N-acetylglucosaminidase, acetyl-CoA: α-glucosaminide N-acetyltransferase, N-acetylglucosaminide-6-sulfate sulfatase, α-N-acetylgalactosaminidase, sialidase, β-glucuronidase, β-hexosaminidase A, and a combination thereof, optionally wherein the lysosomal enzyme comprises at least one asparagine (N)-linked glycan.

15. The method of claim **8**, wherein the glycan oxazoline comprises at least one Man6Pa1,2Man moiety,



16. The method of claim **8**, wherein the glycan oxazoline is a disaccharide oxazoline, trisaccharide oxazoline, tetrasaccharide oxazoline or pentasaccharide oxazoline.

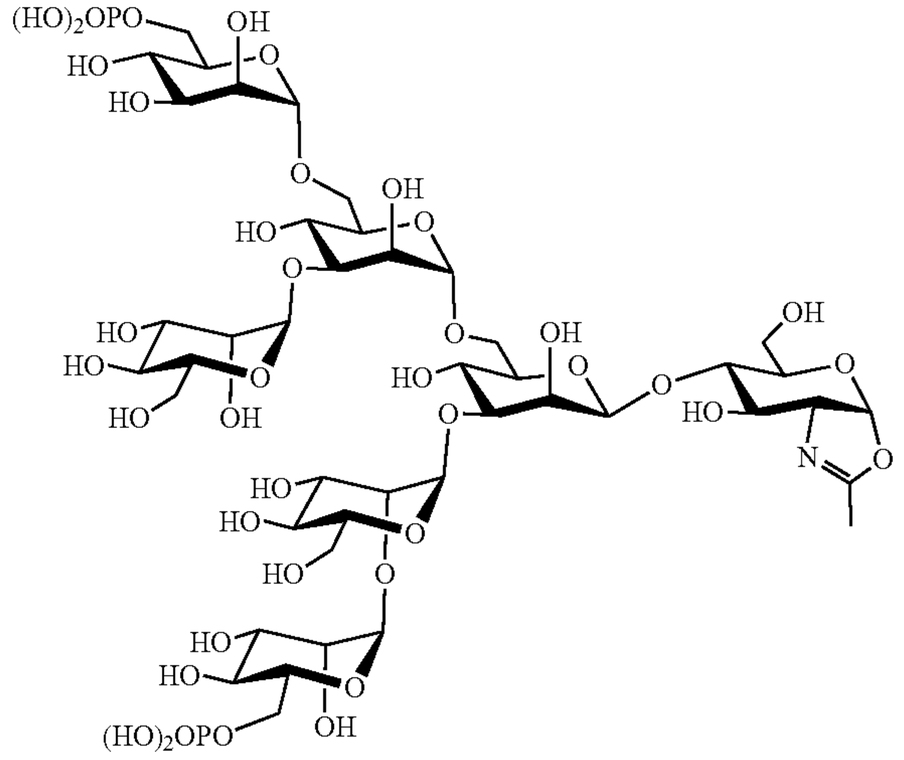
17. The method of claim **8**, wherein the glycan oxazoline has a structure of formula (I), or a salt thereof,



(I)

wherein G is bond or a linker, optionally wherein the linker is a sugar moiety.

18. The method of claim **8**, wherein the glycan oxazoline is selected from the group consisting of



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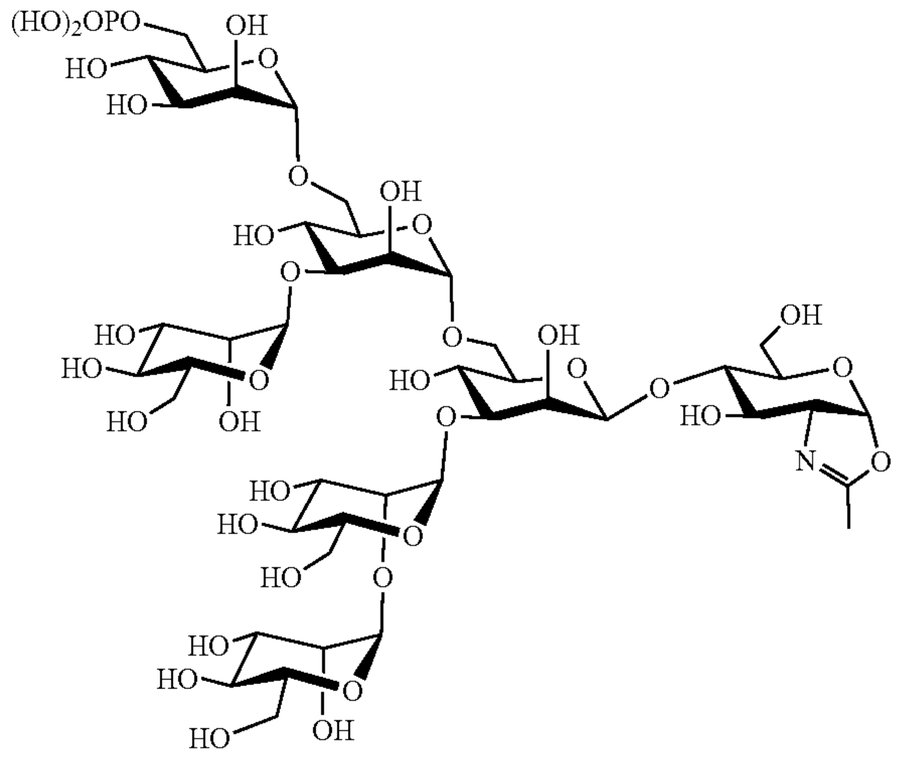

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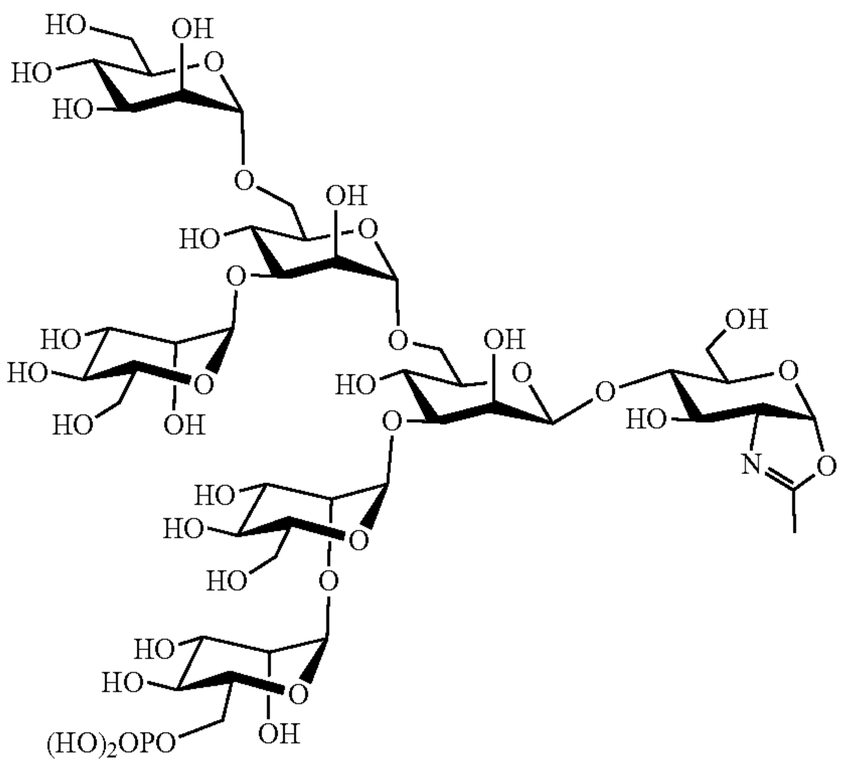

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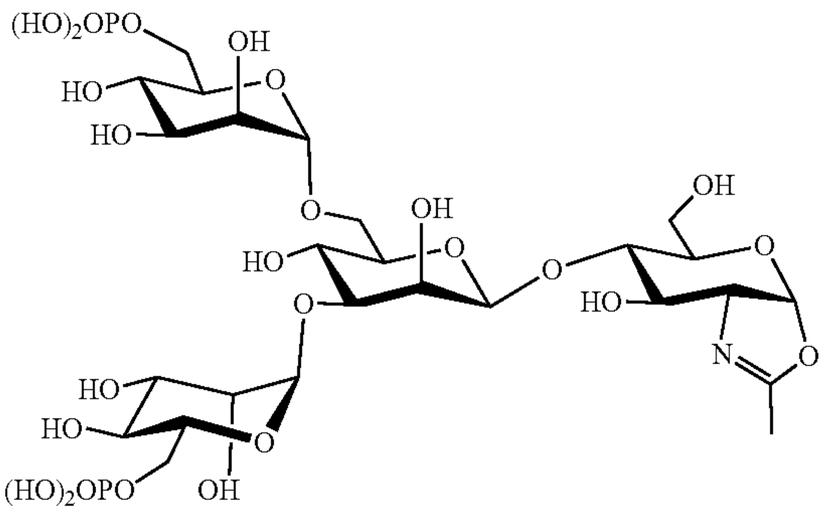

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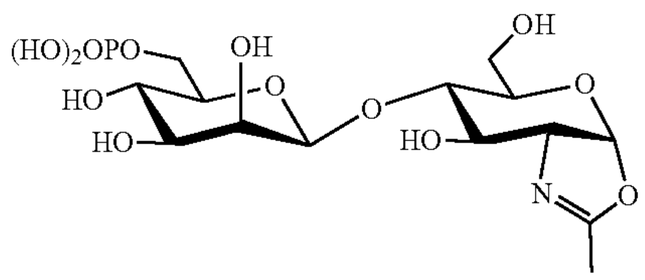

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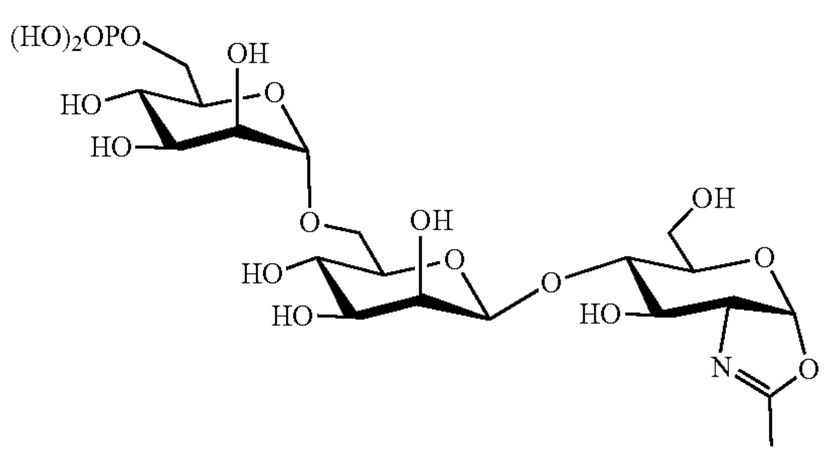

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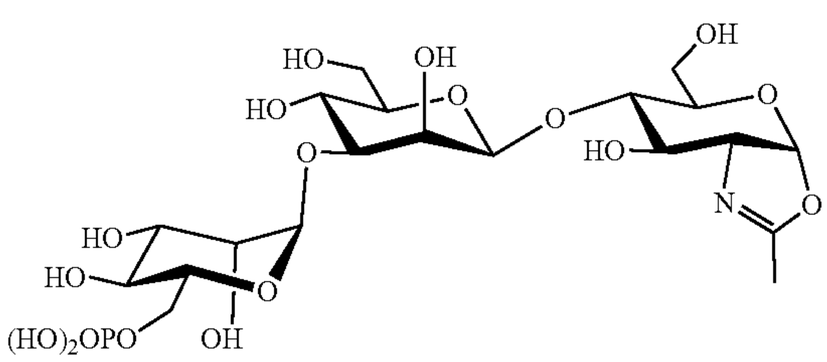

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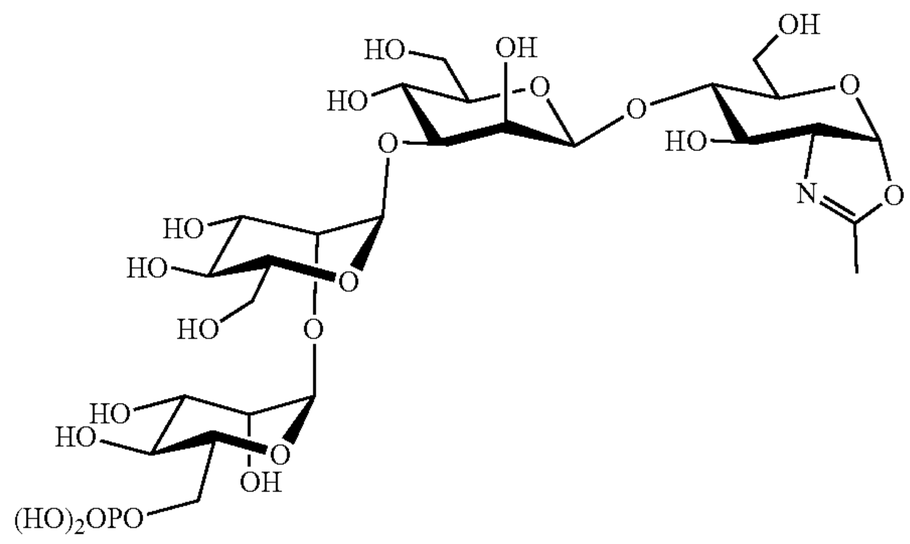

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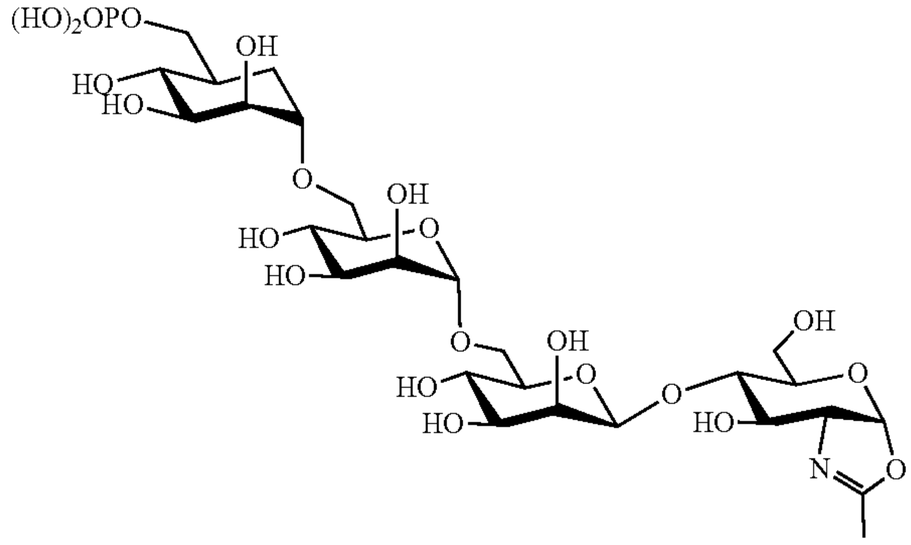

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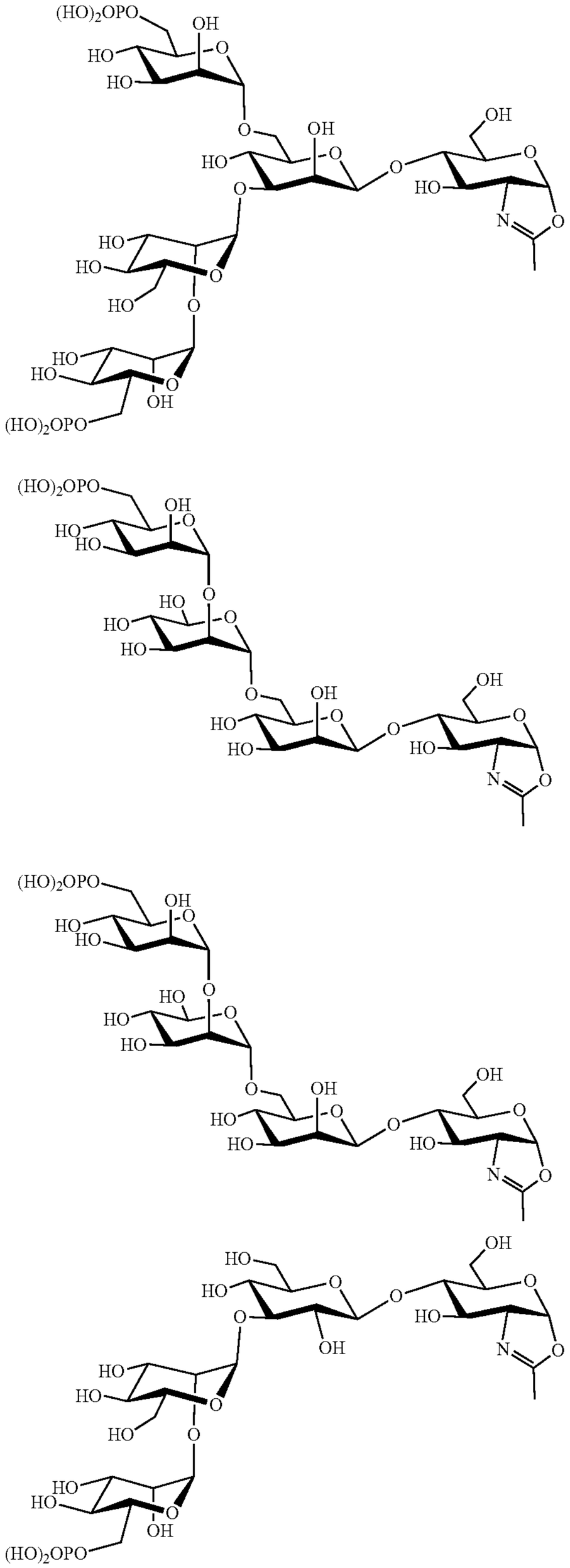

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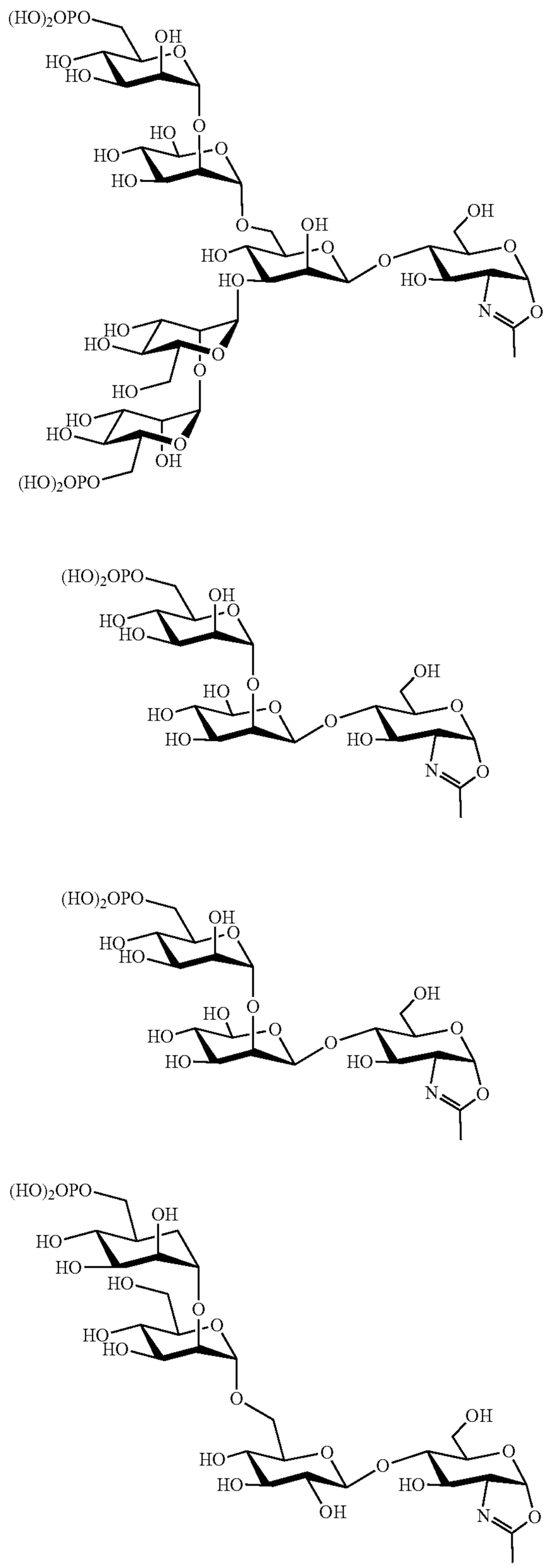
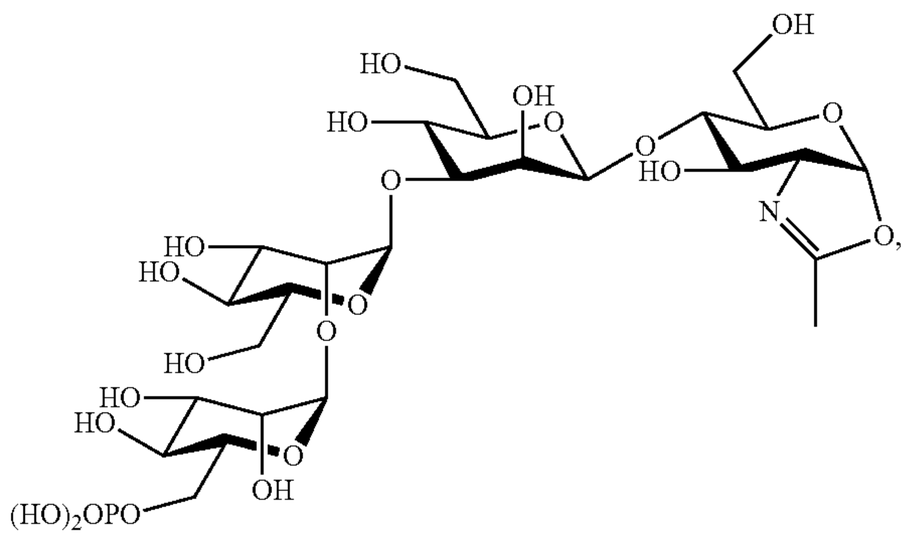


or a salt thereof.

19. The method of claim **8**, wherein the glycan

8



oxazoline is or a salt thereof.

20. A method of enhancing binding affinity of a glycoprotein to a cation-independent M6P receptor (CI-MPR), comprising remodeling the glycoprotein according to the method of claim **8** and contacting the glycoprotein with a cell comprising CI-MPR receptor, thereby enhancing binding affinity of the glycoprotein to the CI-MPR.

21. A method of enhancing or increasing uptake of a glycoprotein in a cell, comprising

(a) remodeling the glycoprotein according to the method of claim **8**, and

(b) contacting the cell with the remodeled glycoprotein, thereby enhancing uptake of the glycoprotein in the cell.

22. (canceled)

23. (canceled)

24. The method of claim **21**, wherein the glycoprotein is acid α-glucosidase (α-glucosidase), and the cell is a muscle cell.

25. A glycan-remodeled glycoprotein produced by the method of claim **8**.

26. (canceled)

27. (canceled)

28. A method of treating Pompe disease in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of the glycan-remodeled lysosomal enzyme of claim **24**.

29. (canceled)

30. (canceled)

* * * * *